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Research Article

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Pollination Bag Type Affects Ovule Development and Seed Yields in *Pinus taeda* L.

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Abstract

Loblolly pine (*Pinus taeda* L.) is the most widely planted forest tree species in the United States. Most of the seedlings used to establish these plantations come from seed collected in open-pollinated seed orchards, but an increasing number are coming from controlled crosses, about 15%–20% of the loblolly pine seedling crops in the last five years. To produce this seed, millions of pollination bags are installed each spring in orchards throughout the southeastern United States; over 2.6 million bags were installed in 2022. This study evaluated 13 pollination bag types available for use in the mass production of control-cross seed. Using cone analysis, significant differences were found among bag types for the proportion of ovules resulting in filled seed, empty seed, and first-year aborts. Due to differences in the efficacy of orchard management, study trees varied greatly in their proportion of ovules resulting in filled seed and first-year aborts. Under good orchard management, open-pollinated cones had 72% of their ovules as filled seed and 12% in first-year aborted ovules. The best pollination bag type had 62% of its ovules as filled seed with 22% in first-year aborted ovules. These differences are apparently due to the quality of pollen used in the controlled crosses.

Study Implications: Compared with open-pollinated families, full-sibling crosses among elite parents of loblolly pine produce more market value to landowners due to greater productivity, increased disease resistance, and enhanced stem form. Specific crosses of loblolly pine have occupied about 15%–20% of the recent seedling market because the seed are costly and difficult to produce. This study tested pollination bag types to determine their effectiveness in producing control-cross seed. Some bag types were superior in increasing seed yield, but seed yields for open-pollinated cones tended to be higher, suggesting problems in the control-cross process. Cone analysis is a useful tool for seed orchard managers to diagnose problems in seed production. Understanding and correcting these problems will help managers increase their production of full-sibling seed and lead to the establishment of new plantations with increased forest productivity.

Keywords: *Pinus taeda*, cone analysis, controlled pollination, seed yield, seed orchard

In the southern United States, loblolly pine (*Pinus taeda* L.) is the most widely used tree species for establishing forest plantations. In the 2020–2021 planting season, over 900 million loblolly pine seedlings were grown in nurseries in the southern United States (Enebak and Newell 2021). Although most of the seedlings planted are with open-pollinated families where the male parent is unknown, about 160 million or 19% of the 2019–2020 loblolly pine seedling crop was composed of control-pollinated families among elite parents (McKeand 2019). Control-pollinated seeds are produced when pollen from a parent is applied to the female strobili of another parent to produce full-sibling seed. The female strobili must be protected from outside pollen contamination using a pollination bag, and the desired pollen is injected into this bag when the female strobili are receptive (Bramlett 1997). Control pollination allows increased genetic gain, since pollen from trees with the best genetic quality are used to combine desirable parental traits, whereas open-pollinated seeds can be contaminated with wild nonimproved pollen from trees outside of the seed orchard. Greenwood and Rucker (1985) used pollen traps and estimated contamination of outside pollen in a loblolly pine seed orchard ranged from 31%–88%, similar to results found by Squillace and Long (1981).

The market for control-pollinated seed has steadily increased over the past 20 years (McKeand 2019) and is projected to increase due to the added genetic gain achieved by crossing superior parents (Bridgwater et al. 1998; McKeand et al. 2021). According to yearly surveys of members of the NC State University Cooperative Tree Improvement Program, there were more than 2.6 million pollination bags installed in the spring of 2022, and over 13.5 million bags have been installed since the annual survey began in 2016 (figure 1).

The mass production of control-pollinated seed is costly and labor intensive (Bridgwater et al. 1998). It tends to yield fewer seeds per cone than open pollination (Snyder and Squillace 1966). Due to the large number of bags installed each year, research has been taking place to evaluate the design of pollination bags to increase control-cross seed production. A recent

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study reported significant differences among different pollination bag prototypes on female strobilus survival (Heine et al. 2020). The addition of a support wire in kraft paper bags or the use of a pollination bag made from stiff material increased female strobilus survival by reducing rubbing against the sides of the bags, which can damage strobili to the point of abortion. The present study uses additional data from Heine et al. (2020) that includes cone analysis and seed yield from the harvested cones.

Cone analysis is a procedure that was developed to help orchard managers evaluate their seed production and diagnose issues in orchard management (Bramlett et al. 1977). Cone analysis involves dissecting individual cones and counting the seed produced compared with their biological potential by counting fertile scales and classifying ovules into filled seed, empty seed, first-year aborted ovules (first-year aborts), and second-year aborted ovules (second-year aborts). This information can help diagnose problems in orchard management and identify solutions for mitigation. This study also included measurements of seed yield from bulked cones, similar to operational processing.

The objectives of this study were to (1) evaluate cone analysis and seed yield measurements from different pollination bag prototypes to determine whether their performance varied due to bag material or design, (2) compare cone analysis and seed yield measurements from open-pollination to those of pollination bags, and (3) understand underlying causes of inefficiencies in cone and seed development when using pollination bags.

Materials and Methods

Experimental Design

The study installations took place in February/March of three separate years: 2014, 2015, and 2017. The bag prototypes were created by PBS International[1] and varied in the material used and bag shape (Supplemental Table S1). The bags evaluated in each study year varied as data were collected about their performance; poor performing bags were removed from further study, and new prototypes were introduced as the study progressed. Branches containing open-pollinated strobili (treatment OP) were tagged at time of bag installation. Bags were pollinated when most female strobili for the tree were at maximum receptivity (Stage 5 of Bramlett and O'Gwynn 1980). Every bag was pollinated twice, usually a few days apart, to pollinate any female strobili that developed at different rates within the bag. Each pollination was done using the same pollen lot on all bags within a particular tree crown. Bags were removed once crews deemed that all female strobili inside each bag were completely closed and were no longer receptive to pollen (Stage 6 of Bramlett and O'Gwynn 1980).

In 2014, six bag types were tested: PBS-A, PBS-B, PBS-C, and PBS-D, a kraft bag with a wire for additional support (Kw), and a kraft bag without a wire (K) (Supplemental Table S1). Nine seed orchards were included in the study. In each orchard, three trees of different genotypes were used. In each tree crown, ten blocks of the treatments were installed, with treatments in the same block grouped closely together in the crown. The OP treatment was included in at least two blocks per tree at all nine seed orchards.

In 2015, nine orchards participated, with three trees of different genotypes in each orchard, but only five blocks were installed per tree. In two blocks per tree, there were nine treatments (PBS-A, PBS-B2, PBS-E, PBS-F, PBS-G, PBS-H, K, Kw, and OP). In three blocks per tree, the PBS-A and PBS-B2 bags were omitted.

In 2017, six orchards participated with three trees of different genotypes per orchard and ten blocks in each tree. There were five treatments within each block: PBS-A, Kw, OP, PBS-A2, and PBS-I2. The PBS-A2 and PBS-I2 were new prototypes that included a flap on the top of the bag that folds down to promote openness at the top of the bag and reduce damage to female strobili during strong wind events, which are typical for the region during spring.

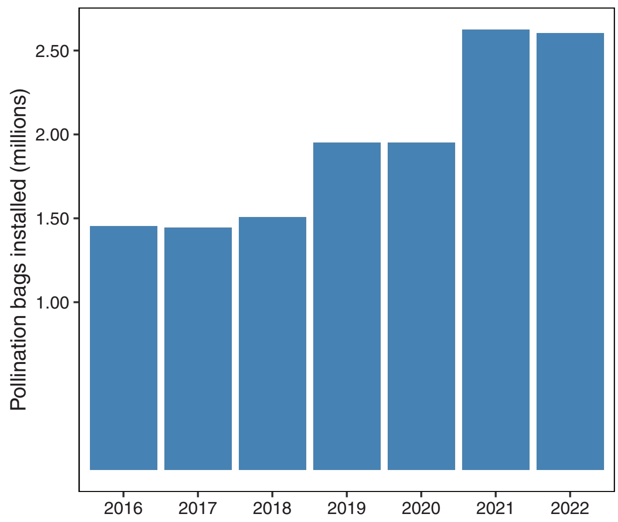

Figure 1. Number of pollination bags installed annually across the southeastern United States since 2016 for mass production of control-cross seed. Data have been collected by the NC State University Cooperative Tree Improvement Program in an annual survey of its full members since 2016.

[1]PBS International can be found here – https://www.pbsinternational.com/ (Last accessed, October 20, 2022)

Measurements

Mature cones were harvested about 18 months after pollination. For the 2014 installation, one healthy cone from each bag was collected from all blocks in each tree. Cones considered healthy did not contain visible insect damage such as holes bored into the cones, presence of frass from insect feeding, dead or distorted cone scales, or scales coated in resin (Ebel et al. 1980), all of which were noted and observed in the harvested cones in all three study years. For the 2015 and 2017 installations, all surviving cones were collected from five blocks per tree. For cone analysis, one healthy cone for each bag type was collected and bagged individually on two blocks in the 2014 and 2015 installations and on three blocks in the 2017 installation. The count of cones used in cone analysis by study year and treatment is given in Supplemental Table S2. The treatments OP, Kw, and PBS-A were common to all study years. For measuring seed yields, seed was extracted from all cones pollinated in 2015 and 2017.

Cones were dried immediately after harvest by spreading them out on wire racks and placing them into a drying chamber set to 37.7°C and 30% relative humidity. Once all cones were completely open (about 10 days in the chamber), they were processed.

Cone Analysis Measurements

A modified protocol for cone analysis was developed following the basic framework as described by Bramlett et al. (1977). Each cone was measured for the length and width at the widest point of the cone. Following measurement, each cone was gently tapped to remove as much seed as possible from the cone. Seed was recorded as either a first-year abort, second-year abort, filled seed (developed ovule that sinks in float test), or empty seed (developed ovule that floats in float test). Our protocol deviated from Bramlett et al. (1977) by using a float test in water to further separate developed seeds as filled or empty (instead of a radiograph).

Next, each cone was placed into a container full of water and soaked for 24 hours until the cone scales had completely closed. Once closed, each cone was dissected. Starting at the base of the cone, each scale was carefully removed from the cone axis with a knife. After the axis was exposed, a drill press was used to drill out the cone axis. Drilling was done using a 9.5 mm brad-point drill bit to allow easy removal of the scales. As scales were removed, they were classified as either fertile or infertile (Bramlett et al. 1977). During dissection, if additional ovules were removed from the cone, these were added to their appropriate category as described above.

Seed Yield Measurements

Only cones collected from the 2015 and 2017 pollinations were used to measure seed yields, as the cones collected from the 2014 pollinations were limited to one healthy/undamaged cone per pollination bag. Cones collected from the 2015 and 2017 pollinations included damaged and unhealthy cones that would not have been eligible for cone analysis. Cones were bulked and processed in batches that corresponded to individual pollination bags and the analogous branches from the OP treatment. Cones were shaken in a plastic container to remove seed. Seed was then rubbed in a cloth to remove the seed wings, blown in a seed blower to separate the chaff, and then float-tested in water. After agitation in the water, the empty seeds were discarded. Seeds that sank were removed from the water, dried, and counted as an observation of seed yield (Bonner and Karrfalt 2008). Cones from the same orchard were processed by the same person to block nuisance variation.

Statistical Analysis

Linear mixed models were used to test for differences among bag types and estimate bag type means. For omnibus hypothesis tests, the OP treatment was removed from the data to focus the inference among pollination bag types. When estimating bag type effects, the OP treatment was included, because its relative performance was of interest. Specific contrasts of interest were bag types K versus Kw (the effect of the support wire) and pollination bags compared with OP.

Control-Pollination Efficiency and Apparent Pollination Effectiveness

Preliminary analysis indicated a large amount of variation in cone analysis and seed yield measurements among orchards and trees within orchards that was apparently due to pollination and/or orchard management quality (e.g., pollen quality, pollination timing, and/or insect protection). The differences in orchard management quality affected the relative performance of the bag type, as trees with poor control-pollination practices tended to have consistently poor cone measurements and seed yield regardless of bag type. To account for differences in the quality of the control-pollination procedure among trees, we created a variable called control-pollination efficiency (CPE). Only cones from control-pollination were included when calculating the trees' CPE (i.e., the OP treatment was excluded). The CPE for a tree was defined as the average seed efficiency, which is the count of filled seed in a cone divided by twice the count of the cone's fertile scales[2] (Bramlett et al. 1977). The CPE was used as a covariate in the statistical models.

The CPE depends on pollination quality (e.g., pollen quality and pollination timing) as well as the protection of developing cones from damage (e.g., insect feeding). To determine the amount of variation in CPE due to pollination quality, we compared the CPE with the apparent pollination effectiveness (APE). The APE was also calculated for control crosses only by dividing the number of developed seeds (both filled and empty) by twice the count of fertile scales (Matthews and Bramlett 1986). The APE is similar to the CPE, but APE includes both filled and empty seed in the numerator, and CPE includes only filled seed. The APE indicates how effectively each tree was pollinated (assuming adequate insect protection). Developed seeds (including empty seeds) are indicators of pollination success, because if one pollen grain germinates inside the pollen chamber, seed coat development is initiated for that ovule (Matthews and Bramlett 1986). A simple linear regression of CPE against APE was done to determine the percent of variation in CPE attributable to pollination quality.

Cone Analysis

The model used for cone length, cone width, the count of fertile scales, and the count of infertile scales (all normally distributed, Supplemental figure S1) was

$$Y_{ijkl} = \mu + B_i + C_{ijk} + BC_{ijk} + O_j + T(O)_{jk} + \varepsilon_{ijkl} \quad (1)$$

where Y_{ijkl} is the cone trait from bag type i, at orchard j, in tree k, and block l; μ is the overall mean; B_i is the fixed bag type effect; C_{ijk} is the CPE covariate; BC_{ijk} is the fixed effect of the interaction between bag type and CPE; O_j is the random orchard effect with expectation $O_j \sim N(0, \sigma_O^2)$; $T(O)_{jk}$ is the random tree nested within orchard effect $\sim N(0, \sigma_{T(O)}^2)$; and ε_{ijkl} is the random error effect $\sim N(0, \sigma_\varepsilon^2)$. Trees that were included in multiple years were treated as distinct because the pollen lot, bag installation crew, and weather varied among years. The block within tree effect was not included in the model because cone collection for cone analysis was permitted from different blocks within a tree when survival was low or insect damage was pervasive.

The counts of ovules classified as filled seed, empty seed, first-year aborts, and second-year aborts were modeled using a generalized linear mixed model with the logistic link function. The ovule counts were considered as the observed number of successes from a binomial distribution with number of trials equal to twice the count of fertile scales, which is their

[2]Bramlett et al. (1977) defines twice the count of fertile scales as a cone's seed potential, as it is the maximum number of ovules that could result in filled seed.

biological maximum. Their distributions are displayed in Supplemental figure S2. A separate model was built for each ovule class as

$$\log(\pi/(1-\pi)) = \mu + B_i + C_{ijk} + BC_{ijk} + O_j + T(O)_{jk} \quad (2)$$

where π is the probability of an ovule being classified as the modeled class, $\log(\pi/(1-\pi))$ is the log transformed odds, and other terms as previously defined.

Model parameters were estimated using the lmer() function for normally distributed responses (cone length, width, and counts of fertile and infertile scales) and the glmer() function for ovule counts from the lme4 package in R (Bates et al. 2015). For the normally distributed responses, the significance of fixed effects was tested with the Type III (conditional) Wald F tests using the Kenwood-Rogers method for approximating the degrees of freedom as implemented in the R package lmerTest (Kuznetsoca et al. 2017). For binomial counts, the analysis of deviance using Type III Wald χ^2 tests were used to test for significance of fixed effects as implemented in the Anova() function of the R package car (Fox and Weisberg 2019).

Least square means were estimated and multiple comparisons were conducted using the Tukey adjustment. Least square means were estimated with the CPE covariate evaluated at a value of 0.55, which was suggested as the minimum value indicative of adequate orchard management by Bramlett et al. (1977) and was near the mean in the data set (0.48). The Kenwood-Roger method was used for estimating degrees of freedom as implemented in the emmeans R package (Lenth 2021). For generalized linear mixed models, multiple comparisons of least square means were performed on the logit scale and then converted to the probability scale for presentation.

Seed Yield

The seed yield measurements were count data with a right-skewed distribution and a large frequency of observations at zero (Supplemental figure S3). Observations of zero seed yield values correspond to bagged branches where all cones aborted, or the harvested cones did not yield seed (e.g., due to insect damage). As a preliminary analysis, the Poisson, negative binomial, and their zero-inflated counterparts were fit with only a term for the mean (intercept) and compared using rootograms (Kleiber and Zeileis 2016). The rootograms indicated that the negative binomial distribution fit the data better compared with Poisson distribution due to overdispersion, and the zero-inflated negative binomial model provided the best fit by better accounting for the high frequency of zero observations (Supplemental figure S4). The zero-inflated negative binomial is a mixture model that models zeroes as coming from two different processes, a binomial process and a count process (Zuur et al. 2009). A logistic regression is used to model the probability of observing a zero (the binomial process). The count process is modeled using a negative binomial generalized linear model with the log link function. The probability mass function is given by

$$f(y_i = 0) = \pi_i + (1 - \pi_i)\left(\frac{k}{\mu_i + k}\right)^k \quad (3a)$$

$$f(y_i | y_i \rangle 0) = (1 - \pi_i) f_{NB}(y) \quad (3b)$$

where y_i is the seed yield from branch i. Equation 3a describes the probability of zero seed yield. The first term in Equation 3a, π_i, is the probability of zero seed yield from the binomial process and is modeled using the logit link as $\pi_i = e^{\nu_i}/(1 + e^{\nu_i})$ where ν_i is the linear predictor containing terms for an intercept, fixed effects, and random effects. The second term in Equation 3a describes the probability that zero seed yield originates from the negative binomial process with mean μ_i and dispersion parameter k. The μ_i is modeled using the log link as $\mu_i = e^{\eta_i}$ where η_i is the linear predictor containing terms for an intercept, fixed effects, and random effects. Equation 3b models the seed yield given that it is greater than zero, where $f_{NB}(y)$ is the probability mass function for the negative binomial with mean μ_i and dispersion parameter k. The expected value of the zero-inflated negative binomial is $E(y_i) = \mu_i(1 - \pi_i)$, which corresponds to the predicted seed yield from the negative binomial process multiplied by the probability that any seed is yielded. The variance of the response is given by

$$\mathrm{Var}(y_i) = (1 - \pi_i)\left(\mu_i + {\mu_i^2}/{k}\right)\mu_i^2\left(\pi_i^2 + \pi_i\right) \quad (4)$$

To determine important factors in explaining variation for seed yield, several combinations of fixed effect variables were evaluated in the linear predictors ν_i and η_i, including count of strobili bagged, CPE, bag type treatment, and their interactions. A random tree effect was included in all models. The Akaike information Criterion (AIC) fit statistic was used to identify the most parsimonious model. The model selection procedure was done with and without the OP treatment, with the former to compare control-cross yields with OP and the latter to determine whether differences existed among pollination bag types for seed yield. Zero-inflated negative binomial mixed models were fit using the mixed_model() function in the R package GLMMadaptive (Rizopoulos 2021). As nested random effects are not implemented in this function, the random effect for tree was not nested within orchard (which assumes that trees from the same orchard are independent).

The seed yield model was developed from observations that included damaged and unhealthy cones. Preliminary analysis found that measurements of seed yield were highly variable and produced much less filled seed per cone than observed in cone analysis. An alternative estimate of seed yield, which we call potential seed yield, was derived using the estimates of fertile scales and proportion of filled seed from the cone analysis models (which only used healthy, damage-free cones) and the strobilus survival probability estimates from the models presented by Heine et al. (2020). We define the potential seed yield as the product of the model estimates (strobilus survival probability, twice the number of fertile scales, and the probability of an ovule developing as filled seed) multiplied by a given number of strobili on the branch during bagging. We compared the predicted seed yield (estimated from data including damaged/unhealthy cones) with the potential seed yield (estimated using healthy cones) to quantify the discrepancies between the two estimates.

Results

Control-Pollination Efficiency versus Apparent Pollination Effectiveness

The APE explained the majority of the variation (62.5%) in CPE (figure 2). The APE indicates how effectively each tree was pollinated, and this was a major factor influencing the efficiency of the control pollinations. Of the 64 study trees, there were only six with a CPE much lower than expected based on their APE.

Cone Analysis

Cone Size

For cone length, the tree effect nested within orchard explained a large amount of variation (Table 1). The orchard effect had an estimate of zero (at the boundary of the parameter space) and was removed prior to hypothesis testing and estimating treatment effects. For cone width, the variation among trees within orchards was also large compared with variation among orchards. For both cone length and cone width, the F test for CPE was significant, whereas its interaction with bag type was not (Table 2). Trees with higher CPE tended to have longer and wider cones (Supplemental figure S1). The main effect for bag type was considered significant for cone length but not for cone width. As a reminder, omnibus tests were used to test the overall significance of bag type and excluded the OP treatment. The OP was added back to the analysis for multiple comparisons testing. The Tukey-adjusted multiple comparisons indicated that PBS-D had significantly shorter mean cone length than Kw, PBS-A, and PBS-E, with differences in estimates ranging from 0.50 to 0.65 cm (Table 3). Multiple comparisons did not detect significant differences in cone width among bag types. Compared with OP, the mean cone length for PBS-D was significantly shorter (by 0.60 cm). Significantly narrower mean cone widths were detected for K, Kw, PBS-A, PBS-D, PBS-E, and PBS-I2 than for OP. The K and Kw were not significantly different for cone length or width.

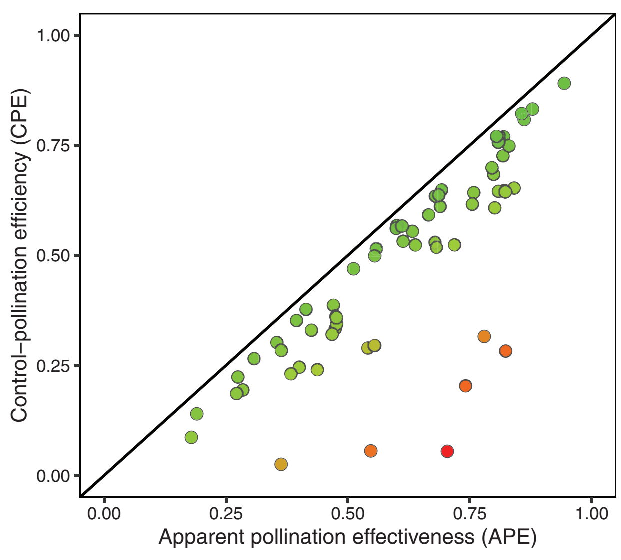

Figure 2. Control-pollination efficiency (CPE) plotted against the apparent pollination effectiveness (APE) for the study trees (black reference line represents parity). A linear regression indicated that most of the variation in control-pollination efficiency (62.5%) was explained by the apparent pollination effectiveness. The control-pollination efficiency values were always lower than apparent pollination effectiveness, indicating the negative influence of factors unrelated to pollen quality or pollination timing. Six trees (red points) were far below the parity line.

Fertile Scales and Infertile Scales

For counts of fertile scales and infertile scales, the orchard variance was less than that of variance due to trees within orchards (Table 1). The orchard variance was zero (fixed at the boundary) for fertile scale counts and excluded from the model. The F tests for bag type, CPE, and their interaction were not significant for the number of fertile or infertile scales (Table 2); however, bag type was marginally significant for the number of fertile scales ($P = 0.07$). The Tukey multiple comparisons (which included the OP treatment and thus had more power) indicated significantly fewer fertile scales for K (mean of 80.14) than for PBS-E (mean of 85.49) (Table 3). The fertile scale count for OP (mean of 84.14) was also significantly more than K. There was no evidence for differences between bags and OP for infertile scale counts, which had a mean of 65.8 (standard error of 4.5). The K and Kw treatment were not significantly different for fertile or infertile scale counts.

Proportion of Ovules

Filled Seed.

For the proportion of ovules resulting in filled seed, the variance among trees within an orchard was greater than between orchards (Table 1). The fixed effects for the bag type, CPE, and their interaction were highly significant (Table 2). As CPE increased from very poor levels (<0.3) to levels indicative of adequate orchard management (>0.55), the filled seed proportion for pollination bags increased, and the differences among pollination bags increased to around 0.70, after which performance among bag types converged (figure 3). The disparity between pollination bags and OP disappeared around a CPE of 0.70, and for CPE greater than 0.75, pollination bags were estimated to outperform OP. The proportion of filled seed for OP was slightly affected by the CPE, indicating that adverse factors unrelated to controlled pollination (such as insect feeding) were present. When conducting multiple comparisons among treatments at a CPE of 0.55, all bags had a significantly lower proportion of ovules as filled seed compared with OP (Table 4). There were several significant differences among bag types, with PBS-I2 having the highest mean proportion of filled seed (0.62) and PBS-H, which was significantly worse than all other bag types, having the lowest estimate (0.49). The K and Kw treatments performed similarly for proportion of filled seed.

Empty Seed.

The variation among trees within an orchard was greater than between orchards for the proportion of ovules resulting in empty seed (Table 1). The fixed effects for bag type, CPE, and their interaction were highly significant (Table 2). The proportion of empty seed for the OP was unaffected by the CPE for a given tree (e.g., the line was essentially flat in figure 3). For CPE less than 0.30, all bags had significantly higher proportions of empty seed than OP, but the disparity subsided at a CPE of 0.55, where PBS-B2 and PBS-I2 had significantly lower proportions of empty seed than OP (Table 4) and the other bag types were not significantly different than OP. PBS-B2 and PBS-I2 also had significantly lower proportions of empty seed compared with K, Kw, PBS-F, and PBS-H. The K and Kw performed similarly for proportion of empty seed.

Table 1. Variance component estimates for normally distributed traits (cone length, cone width, and counts of fertile and infertile scales) and binomial distributed traits (proportion of ovules as filled seed, empty seed, first-year aborts, and second-year aborts). The open-pollinated treatment was excluded. For all traits, variation among orchards was less than variation among trees within orchards. Orchard to orchard variation was zero for cone length and count of fertile scales.

Effect	Orchard	Tree (orchard)	Residual
Cone length	0	1.49 (0.28)	0.64 (0.03)
Cone width	0.01 (0.07)	0.73 (0.15)	0.47 (0.03)
Fertile scales	0	97.18 (18.52)	61.78 (3.25)
Infertile scales	41.68 (27.24)	74.03 (15.81)	50.86 (2.68)
Filled seed	0.013 (0.009)	0.039 (0.009)	-
Empty seed	0.175 (0.124)	0.538 (0.107)	-
First-year aborts	0.111 (0.073)	0.281 (0.056)	-
Second-year aborts	0.015 (0.062)	0.501 (0.106)	-

Table 2. Wald tests for bag type, control-pollination efficiency (CPE), and their interaction for normally distributed traits (cone length, cone width, and counts of fertile and infertile scales) and analysis of deviance χ^2 for the binomial distributed traits (proportion of ovules as filled seed, empty seed, first-year aborts, and second-year aborts). The OP treatment was excluded. The main effect of CPE was significant for cone length and width, with higher CPE resulting in larger cones. The effect of bag type was significant for cone length and marginally significant (P = 0.073) for count of fertile scales. All terms were significant for proportion of ovules as filled seed, empty seed, first-year and second-year aborts, except the CPE for second-year aborts.

Source of variation	Bag type	*P*	CPE	*P*	Bag type * CPE	*P*
	Test statistic		Test statistic		Test statistic	
Cone length	1.50	0.010	8.44	0.006	1.21	0.275
Cone width	1.05	0.399	10.12	0.003	0.47	0.933
Fertile scales	1.65	0.073	1.51	0.223	0.75	0.698
Infertile scales	1.18	0.291	0.5	0.482	1.01	0.437
Filled seed	143.65	<0.001	731.92	<0.001	110.55	<0.001
Empty seed	57.34	<0.001	23.79	<0.001	84.35	<0.001
First-year aborts	222.07	<0.001	67.34	<0.001	170.80	<0.001
Second-year aborts	95.15	<0.001	0.05	0.819	123.38	<0.001

Table 3. Least square means (standard errors) of cone size traits for pollination bag types and the open-pollinated (OP) treatment. Letters indicate groupings from Tukey-adjusted multiple comparisons. The OP cones were significantly bigger and had higher counts of fertile scales than some pollination bags. The count of infertile scales did not differ among treatments.

Treatment	Cone length (cm)	Cone width (cm)	Count of fertile scales
OP	10.22 (0.17)^{a}	6.87 (0.12)^{a}	84.14 (1.39)^{a}
K	10.03 (0.18)^{ab}	6.46 (0.13)^{b}	80.14 (1.49)^{b}
Kw	10.27 (0.17)^{a}	6.43 (0.12)^{b}	83.71 (1.39)^{ab}
PBS-A	10.12 (0.17)^{a}	6.57 (0.12)^{b}	82.22 (1.40)^{ab}
PBS-A2	10.08 (0.21)^{ab}	6.54 (0.16)^{ab}	83.55 (1.83)^{ab}
PBS-B	9.92 (0.19)^{ab}	6.53 (0.15)^{ab}	82.26 (1.68)^{ab}
PBS-B2	10.08 (0.21)^{ab}	6.51 (0.16)^{ab}	81.98 (1.81)^{ab}
PBS-C	10.00 (0.20)^{ab}	6.72 (0.15)^{ab}	83.07 (1.69)^{ab}
PBS-D	9.62 (0.19)^{b}	6.45 (0.15)^{b}	81.71 (1.68)^{ab}
PBS-E	10.27 (0.20)^{a}	6.43 (0.15)^{b}	85.49 (1.77)^{a}
PBS-F	10.08 (0.21)^{ab}	6.44 (0.16)^{ab}	83.78 (1.80)^{ab}
PBS-G	9.97 (0.20)^{ab}	6.66 (0.15)^{ab}	81.70 (1.77)^{ab}
PBS-H	10.05 (0.21)^{ab}	6.50 (0.16)^{ab}	83.41 (1.80)^{ab}
PBS-I2	10.00 (0.21)^{ab}	6.35 (0.16)^{b}	81.85 (1.83)^{ab}

First-Year Aborts.

Ovules that abort during the first growing season are believed to be caused by insect damage, lack of pollen, or pollen with a low viability (Bramlett 1993). For the proportion of ovules resulting in first-year aborts, the variation among trees within an orchard was greater than between orchards (Table 1). The

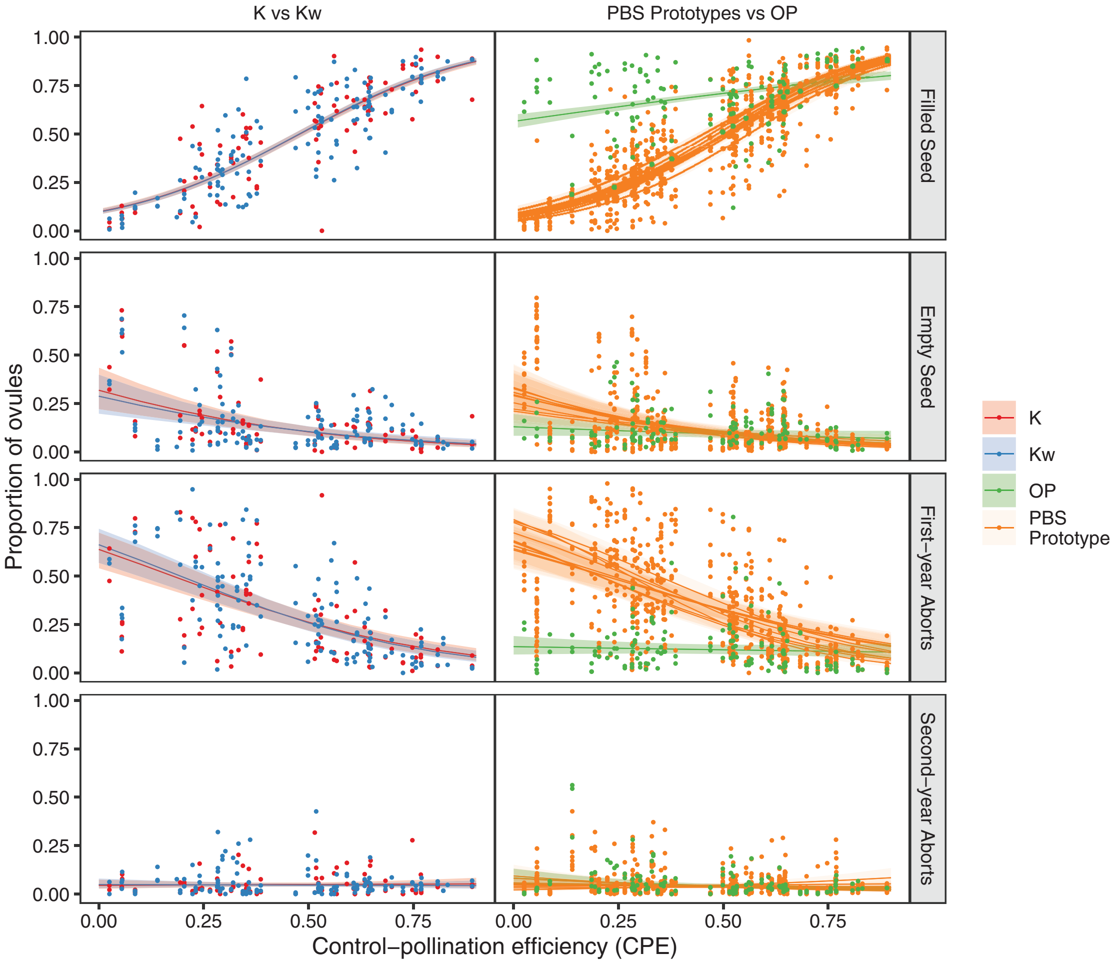

Figure 3. Proportion of ovules resulting in filled seed, empty seed, and first/second-year aborts against control-pollination efficiency by bag type. Lines are the least square means, bands are the 95% confidence intervals, and points are observations. Proportion of filled seed, empty seed, and first-year aborts varied greatly with control-pollination efficiency for pollination bags. All bags performed worse than OP unless the control-pollination efficiency was greater than 0.7. Not all PBS prototypes performed similarly for filled seed and first-year aborts. The kraft (K) and kraft with wire (Kw) performed similarly to each other (plotted separately for visibility).

fixed effects for the bag type, CPE, and their interaction were highly significant (Table 2). For pollination bags, the proportion of first-year aborts dropped substantially with increasing CPE, indicating that first-year aborts were the primary source of loss in seed potential (figure 3). The proportion of first-year aborts for OP was unaffected by the CPE for a given tree (e.g., the line was essentially flat in figure 3). For CPE values less than 0.65, all bag types had a significantly higher proportion of first-year aborts than OP. At a CPE of 0.55, there were significant differences among bag types for proportion of first-year aborts, with PBS-A2 having the lowest mean proportion (0.21) and PBS-H having the highest (0.32) (Table 4). The K and Kw were not significantly different from each other.

Second-Year Aborts.

Ovules that abort in the second year are believed to be a direct indication of seed bug feeding (Bramlett et al. 1977). The proportion of ovules resulting in second-year aborts also varied more among trees within an orchard than among orchards (Table 1). The fixed effects for the bag type and its interaction with CPE were highly significant, but the main effect for CPE was not (Table 2). The proportion of second-year aborts was low for all CPE values for pollination bags and OP (figure 3). At a CPE of 0.55, there were some significant differences among bag types, with PBS-F having the lowest mean proportion of second-year aborts (0.03) and K having the highest (0.05) (Table 4). Bag types K and PBS-D had significantly higher mean proportion of second-year aborts than OP (0.05 versus 0.04). The K and Kw performed similarly.

Seed Yield

When excluding the OP treatment, the seed yield model with the best fit had fixed effects for CPE and number of strobili bagged in both the zero part and the count part of the model (Supplemental Table S3, model 8). Notably, the bag type effect did not improve model fit, indicating that bag type did not explain significant variation in the seed yield data. When including OP, the preferred seed yield model included fixed effects for treatment, CPE, and number of strobili in the zero part, and the count part included the interaction of treatment and CPE as well as the main effect for count of strobili (Supplemental Table S3, model 2). Thus, the treatment effect on seed yield was apparently due to differences between pollination bags and OP and not due to differences among pollination bag types. The significant interaction between CPE and treatment can also be attributed to the OP, because the

Table 4. Least square means (standard errors) of proportion of ovules as filled seed, empty seed, and first/second-year aborts for pollination bag types and open-pollinated (OP). Values are estimates at a control-pollination efficiency of 0.55. Letters indicate groupings from Tukey-adjusted multiple comparisons. The OP cones had more filled seed and fewer first-year aborts than all bag types. Not all bag types performed similarly. The K and Kw treatments were not significantly different.

Treatment	Proportion of ovules			
	Filled seed	Empty seed	First-year aborts	Second-year aborts
OP	0.72 (0.01)[a]	0.09 (0.01)[ab]	0.12 (0.01)[d]	0.04 (0.01)[bcd]
K	0.58 (0.01)[cde]	0.09 (0.01)[ab]	0.23 (0.02)[c]	0.05 (0.01)[a]
Kw	0.58 (0.01)[d]	0.09 (0.01)[a]	0.23 (0.02)[c]	0.05 (0.01)[ab]
PBS-A	0.60 (0.01)[bc]	0.08 (0.01)[bc]	0.22 (0.02)[c]	0.04 (0.01)[abcd]
PBS-A2	0.61 (0.01)[bc]	0.08 (0.01)[abc]	0.21 (0.02)[c]	0.04 (0.01)[abcd]
PBS-B	0.56 (0.01)[def]	0.08 (0.01)[bc]	0.28 (0.03)[ab]	0.04 (0.01)[cd]
PBS-B2	0.56 (0.01)[def]	0.07 (0.01)[c]	0.29 (0.03)[ab]	0.04 (0.01)[bcd]
PBS-C	0.57 (0.01)[def]	0.09 (0.01)[abc]	0.26 (0.02)[b]	0.03 (0.01)[cd]
PBS-D	0.58 (0.01)[bcde]	0.09 (0.01)[abc]	0.21 (0.02)[c]	0.05 (0.01)[a]
PBS-E	0.54 (0.01)[f]	0.08 (0.01)[abc]	0.30 (0.03)[a]	0.04 (0.01)[bcd]
PBS-F	0.54 (0.01)[f]	0.09 (0.01)[ab]	0.29 (0.03)[ab]	0.03 (0.01)[d]
PBS-G	0.55 (0.01)[ef]	0.08 (0.01)[abc]	0.30 (0.03)[ab]	0.04 (0.01)[abcd]
PBS-H	0.49 (0.01)[g]	0.10 (0.01)[ab]	0.32 (0.03)[a]	0.05 (0.01)[abc]
PBS-I2	0.62 (0.01)[b]	0.07 (0.01)[c]	0.22 (0.02)[c]	0.04 (0.01)[abcd]

effect of CPE on seed yield from OP branches is much less than for pollination bags. Predictions from the seed yield model including OP are displayed in a contour plot (figure 4). The data used to build the model are included as points. For pollination bags with eight female strobili bagged (the average number bagged in this study), the CPE must be at least 0.55–0.60 (depending on bag type) for the predicted seed yield to be 300 or more. The predictions of seed yield for OP were only slightly influenced by the CPE and were more than 300 for four to six strobili. At eight female strobili bagged and a CPE of 0.55, the pollination bag types had predicted seed yields that ranged from 273 to 312, with PBS-A, PBS-E, and PBS-I2 performing the best and PBS-G and PBS-H performing the worst. The Kw had a prediction of 300 seed while K had a prediction of 281.

The predicted seed yields are compared with the potential seed yield (estimated using healthy cones from cone analysis) in Table 5. The predictions from the seed yield model (measured from bulked cone collections that included damaged and unhealthy cones) were correlated with the potential seed yields (estimated from cone analysis using healthy cones only) ($r^2 = 0.85$ including OP; $r^2 = 0.68$ excluding OP), but they were consistently lower and varied less among bag types. At a CPE of 0.55 and eight strobili bagged, the potential seed yield estimates ranged from 423.9 to 600.2 among pollination bag types.

Discussion

Orchard management quality, as measured by the CPE, significantly influenced almost all of the traits measured. Trees with poorer control-pollination efficiency had smaller cones, lower proportions of filled seed, and lower seed yields. At low CPE, all pollination bag types performed poorly. The proportion of filled seed for OP cones was slightly influenced by a tree's CPE, indicating the presence of adverse factors such as insect damage, diseases, physiological stress, or genetic embryo incompatibility (Matthews and Bramlett 1983). The impact of CPE on cone length and cone width is presumably due to cones growing larger to accommodate the space needed for filled seed, which are larger in size than aborted ovules. Evidence to support this claim can be found in Nel et al. (2003) as it is mentioned that cones in their no-pollination treatments and self-pollination treatments were noticeably smaller in size than cones that were open-pollinated or pollinated with 50% viable pollen. Bramlett (1993) also observed smaller cones due to stunting in cone scales with nonpollinated ovules. Around half of the trees in this study would fail the criteria that Bramlett et al. (1977) refer to as good orchard management (CPE >0.55).

The low CPE observed in this study could mostly be attributed to APE (apparent pollination effectiveness) (figure 2), indicating that pollination quality (pollen quality or timing of pollen application) was the major factor in explaining the successful conversion of embryos to filled seed in this study. Only six out of the 64 study trees had an APE much higher than the CPE. These results highlight that orchard managers must ensure that cones are pollinated with viable pollen at the proper stage of female strobilus development, and they must protect their cone crop from insect damage.

For all cone analysis measurements, trees within an orchard varied more than trees among orchards (Table 1). Variation among trees for cone length, width, and number of fertile scales was expected due to recognizable differences among clones during cone processing. Differences in the number of fertile scales among loblolly pine clones have been described before (Bramlett et al. 1974, 1977; Karrfalt and Belcher 1976; March et al. 1994). Differences among trees in their ovule breakdown was persistent even after controlling for CPE, suggesting that some trees are better at converting ovules to filled seed, presumably a clonal effect.

Differences among bag types were found in the proportion of ovules developing as filled seed (when the CPE was in the range of 0.3 to 0.7) (figure 3). Nel et al. (2003) found differences among bag type material (white microfiber was superior to green microfiber, polythene, and sponge) for number of developed seeds. In their study, bags made of breathable material (white and green microfiber) also had increased cone survival over polythene-based materials. The suspected reason for this was that cones inside of the breathable material were subjected to an environment similar to ambient air for temperature and humidity, which was not the case for bags composed of cellulose and polythene materials. Our study suggests that bag design and shape along with the type of material used is important. The bags with the highest proportion of filled seed were made of polypropylene (PBS-I2) or a proprietary material which has layers of polyester (PBS-A and PBS-A2).

The shape of the bags also appears important, as bags with the top flap folded down to promote bag openness had the highest proportion of ovules that developed filled seed (PBS-A2 and PBS-I2) (Table 4). These bags were also found to increase strobilus survival (Heine et al. 2020) and ranked high for seed yield (Table 5). The increased proportion of filled seed could be due to a more effective pollination from increased air circulation in bags that are more open. The low proportion of first-year aborts for this bag design supports this explanation, but future studies would need to count the number of pollen grains present per ovule shortly after pollination to confirm this. We also found evidence that bag types affected cone length and number of fertile scales. The bag type differences in number of fertile scales was surprising, as this has previously been believed to be controlled by the tree genotype (Bramlett et al. 1974). It is not obvious to us what led to these bag type differences in cone length and number of fertile scales; however, it is possible that bag type effects on pollination efficiency within a bag could have led to the differences seen in number of filled seed and first-year aborts, which in turn could have affected cone size and scale development. Bag type differences in proportion of second-year aborts were small and unexpected as second-year aborts are believed to be caused primarily by insect feeding (Bramlett et al. 1977).

The K and Kw treatments performed similarly for all cone analysis measurements (Table 3 and Table 4), indicating that addition of the support wire did not affect the development of ovules in paper bags. The only notable difference between K and Kw was in the predictions of seed yield (Table 5). Depending on what method was used, Kw was expected to yield 18.4 to 87.9 more seed per bag than K when bagging eight female strobili at a CPE of 0.55. This can be attributed to the higher strobilus survival probability for bag type Kw versus K as reported in Heine et al. (2020). This suggests that the addition of the support wire contributes to seed production through protection of strobili (e.g., during wind events) rather than affecting the pollination efficiency within the bag.

Except at the upper end of the range for CPE, the OP cones had considerably higher proportions of filled seed (figure 3) and higher seed yield (Table 5) than all pollination bags. Higher seed yields in OP compared with control-pollinated cones has been documented for years in loblolly pine (e.g., Snyder and Squillace 1966). Our cone analysis results indicate

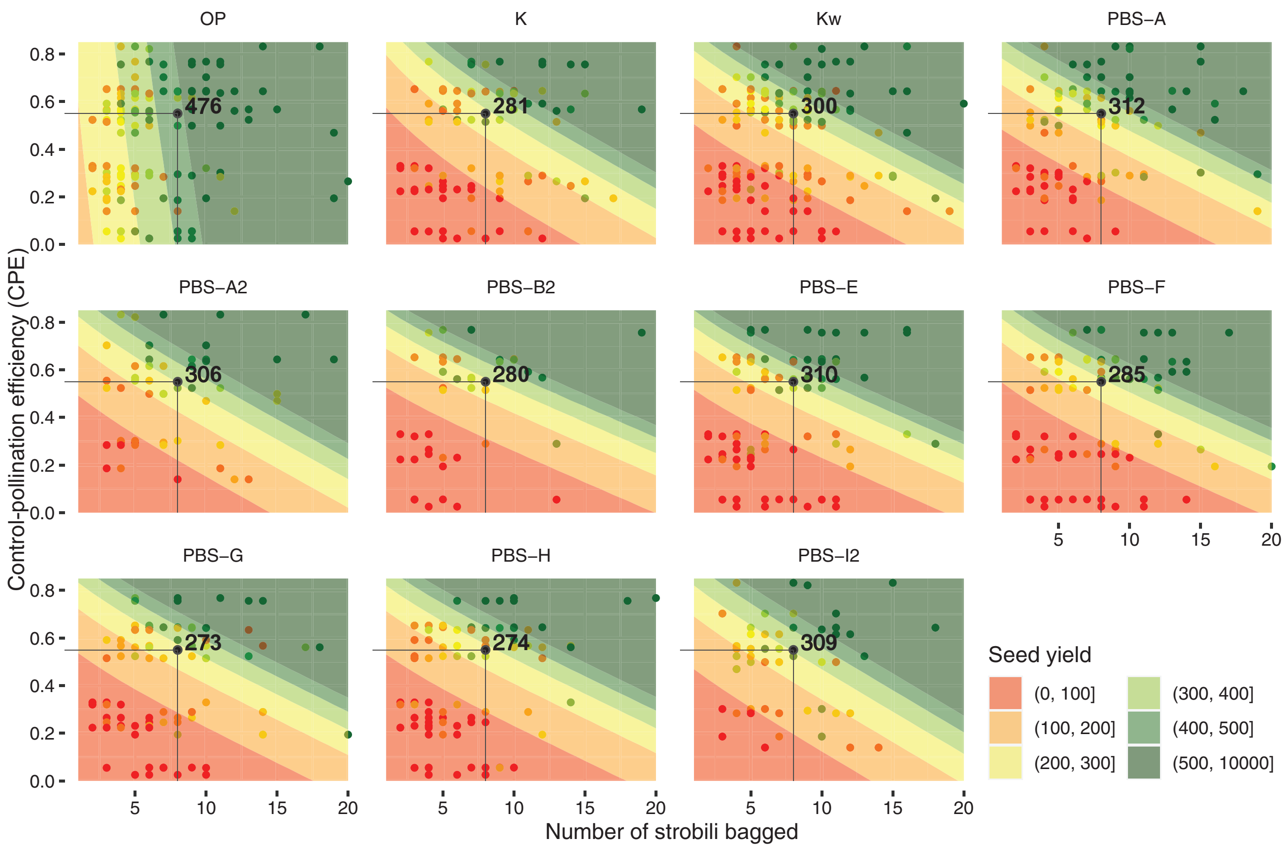

Figure 4. Contour plot of predicted seed yield (green area corresponds to high yield and red for low yield) and observed seed yield (colored points) by number of strobili bagged and the control-pollination efficiency for different bag types using a zero-inflated negative-binomial model. The black reference point corresponds to the predicted seed yield at eight strobili bagged and a control-pollination efficiency of 0.55. Seed yield increased with the number of strobili bagged and the control-pollination efficiency. Open-pollinated cones reached 500 filled seeds with fewer strobili.

Table 5. Seed yield estimates for eight strobili bagged and control-pollination efficiency of 0.55 from two sources: the predicted seed yield (using data from bulked collections that included damaged/unhealthy cones) and the potential seed yield (obtained from cone analysis of healthy, damage-free cones and strobilus survival estimates). The rankings of the bag types were consistent between the two sources, but the estimates were much lower for the predicted seed yield and the differences among treatments were less pronounced.

Treatment	Predicted seed yield (95% CI)	Strobilus survival (SE)	2*Fertile scales (SE)	Filled seed proportion (SE)	Potential seed yield
OP	476 (399, 570)	0.82 (0.03)	169 (2.8)	0.72 (0.01)	798
K	281 (215, 352)	0.57 (0.06)	161 (3.0)	0.58 (0.01)	424
Kw	300 (254, 361)	0.65 (0.05)	168 (2.8)	0.58 (0.01)	512
PBS-A	312 (254, 378)	0.73 (0.05)	165 (2.8)	0.60 (0.01)	576
PBS-A2	306 (239, 389)	0.72 (0.05)	167 (3. 7)	0.61 (0.01)	592
PBS-B2	280 (210, 377)	0.65 (0.06)	164 (3.6)	0.56 (0.01)	479
PBS-E	310 (255, 379)	0.68 (0.05)	171 (3.6)	0.54 (0.01)	508
PBS-F	285 (226, 341)	0.68 (0.05)	168 (3.6)	0.54 (0.01)	492
PBS-G	273 (212, 343)	0.67 (0.05)	164 (3.5)	0.55 (0.01)	484
PBS-H	274 (220, 343)	0.68 (0.05)	167 (3.6)	0.49 (0.01)	443
PBS-I2	309 (248, 386)	0.74 (0.05)	164 (3.7)	0.62 (0.01)	600

that a large portion of the difference can be attributed to first-year aborts. Open-pollinated cones had a significantly lower proportion of first-year aborts (mean of 0.12) than all bag types (mean ranged from 0.21 to 0.32). Nel et al. (2003) also found open-pollinated cones had fewer first-year aborts and more developed seeds compared with pollination bags in *P. patula,* except for one bag type, a white microfiber material, which was not significantly different than open-pollinated cones in number of developed seeds. Ovules that abort during the first growing season are believed to be caused by insect damage, lack of pollen, or pollen with a low viability (Bramlett 1993). If no pollen grains are present in the pollen chamber, or if they all fail to germinate, the ovule will abort in the first year (Bramlett et al. 1977). Insect damage is not likely to have caused differences in first-year aborts between open- and control-pollinated cones, as they were collected from the same study trees, and we do not expect that insects would target control-pollinated cones differently than open-pollinated. Further, unlike the control-pollinated cones, the proportion of first-year aborts for open-pollinated cones was unaffected by CPE. The more likely explanation for higher first-year aborts in control-pollinated cones is a lack of pollen or the use of pollen with low viability.

When female strobili are receptive to pollen, before the scales swell shut, only a small number of pollen grains can enter each ovule's pollen chamber. Bramlett and Matthews (1983) dissected conelets of *P. taeda* and found when female strobili are maximally receptive to pollen (Stage 5), they had an average of 3.7 pollen grains per ovule in controlled pollinations compared with 3.8 pollen grains per ovule for open-pollinated cones. Matthews and Blalock (1981) similarly found that open-pollinated cones had a mean of 4.0 grains per ovule with a range of 0–7 grains. They also found that the two upper-most and lower-most fertile scales averaged roughly 30% fewer grains than scales from the central portion of the conelet. Bramlett et al. (1985) found that when applying 0.25, 0.50, or 1.0 cc of pollen, there was an average of 2.2 pollen grains per ovule, which increased slightly to 2.8 pollen grains per ovule when 2 cc pollen was applied. Open-pollinated ovules had an average of 4.0 pollen grains per ovule. They attributed the lower mean pollen counts per ovule for control-pollinated cones to inadequate distribution of pollen to the ovule or improper timing of pollen application. Moody (1988) dissected *P. taeda* strobili two weeks after pollination and found that open-pollinated conelets had an average of 4.2 pollen grains per ovule, whereas control-pollinated averaged 2.7 grains per ovule. Ten or more grains were found in some ovules, mostly in open-pollinated strobili. These findings stress the importance of pollination timing, pollen distribution, and pollen viability, as a higher number of pollen grains per ovule and pollen with better viability increases the chances of at least one pollen grain germinating to create a developed seed.

We suspect that pollen quality (rather than pollination timing or insect damage) likely explains most of the difference seen in number of first-year aborts between pollination bags and OP in this study. Although variation in flower stages within a bag can make it difficult to time pollination when all female strobili are receptive, the bags in this study were pollinated twice with close monitoring of strobilus development. It seems more likely that, for the ovules that aborted during the first year, the pollen grains did not germinate to stimulate seed coat development, leading to a high number of first-year aborts.

The higher proportion of empty seeds observed in pollination bags at poor control-pollination efficiencies (<0.3) (figure 3) also adds evidence that pollen quality could be the cause for the lack of filled seed in controlled pollinations compared with OP. Empty seeds are thought to be caused by insect feeding, fungi, embryonic lethal alleles, and low pollen vigor (Bramlett 1993). Bramlett (1993) and Bramlett et al. (1985) indicate that pollen can germinate in vivo and stimulate seed coat development but may lack the vigor to complete fertilization and produce gametophyte tissue required for filled seed. Fungi and insect feeding are not expected to have differentially affected control-pollinated cones compared with OP in this study, as the treatments were located on the same trees and oftentimes the same branches. Embryonic lethal alleles are associated with inbreeding, which was not permitted in this study. Low pollen vigor of the lots used is a more likely source of empty seed in this study. Matthews and Bramlett (1983) found that pollen stored in a desiccator for >1 year produced a similar number of developed seeds as fresh and vacuum stored pollen but a much lower number of filled seeds despite comparable in vitro germination tests. Bramlett (1993) describes the "pollination effect" as the scenario where open-pollinated cones have a lower number of empty seeds and first-year aborts compared with control-pollinated cones. To add further evidence, the six of the 64 study trees that had an APE much higher than the CPE (red points in figure 2) were further investigated, and it was found that they came from the same seed orchard across two study years (three trees in 2014 and three in 2015). Control-pollinated cones from these trees had an average CPE of 14% and averaged 22 filled seeds, 81 empty seeds, 43 first-year aborts, and eight second-year aborts per cone. Open-pollinated cones on these same trees had an average seed efficiency of 75% and averaged 127 filled seed, 23 empty seed, 13 first-year aborts, and four second-year aborts per cone. At this seed orchard, poor pollen quality was certainly to blame for low CPE in 2014 and 2015. The high number of developed seeds (filled + empty) suggests that pollen was applied at the correct time and stimulated seed coat development; however, poor-quality pollen led to a higher number of first-year aborts and a much higher number of empty seeds per cone than the open-pollinated cones. Although some pollen grains likely did not germinate (due to low pollen viability), leading to first-year aborts, the majority of pollen grains that did germinate had low vigor and did not complete fertilization, leading to the high number of empty seeds per cone for control-pollinated cones.

The predicted seed yields (Table 5) were much lower than the potential seed yields estimated from cone analysis and the strobilus survival estimates from Heine et al. (2020). The cone analysis methods used a complete seed extraction procedure and excluded cones with excessive insect damage. The direct measurements of seed yield in this study had less intensive seed extraction, as multiple cones were processed at a time and cones were not picked entirely clean, which is more similar to an operational cone extraction processes. The direct seed yield measurements also included cones that were visibly damaged by insects or case-hardened, which tend to release fewer seeds. The pollination bag types differed more in their estimates of potential seed yield than in their predicted seed yield. This is likely due to greater precision of the measurements in cone analysis and strobilus survival used to derive potential seed yield. The measurements used in the predicted seed yields were highly variable, which dilutes the variation that can be attributed to the bag type treatments. Further, they required more complicated modeling. We recommend using the predicted seed yields to make conservative comparisons among bag types and using the potential seed yield estimates to set seed yield goals for what could be produced under ideal orchard management. The highest-ranking bag types were common to both methods. The PBS-I2 had the highest strobilus survival from time of bagging to cone harvest (Heine et al. 2020), and cone analysis indicated it had the highest proportion of filled seeds per cone (Table 5). Bag types PBS-A and PBS-A2 followed closely behind for both strobilus survival and proportion of filled seeds and also ranked well in their predicted seed yield. When compared with the Kw, the PBS-A, A2, and I2 all had significantly higher proportions of filled seeds per cone from cone analysis and higher predictions of seed yield, although the 95% confidence intervals were overlapping (Table 5).

Another consideration is the time and effort required for bag installation, which was found to be greater for the Kw than PBS prototypes by Heine et al. (2020). Orchard managers should consider these findings as well as the bag costs to determine which bag is most appropriate for their orchards.

Conclusions

Pollination bags had considerable variation in the proportion of ovules resulting in filled seed when orchard trees had adequate management. A large number of orchard trees failed to meet the 0.55 CPE threshold recommended by Bramlett et al. (1977), apparently due to poor pollen viability and/or vigor. Open-pollinated cones produced a much lower proportion of first-year aborts than cones from pollination bags. Open-pollinated cones also had a higher proportion of filled seeds and lower proportion of empty seeds, except on a few trees with exceptional CPE.

This study demonstrates that cone analysis is a useful tool for orchard managers to diagnose problems in seed production. Considerable effort has gone into the study of conifer pollen and pollen tubes (Fernando et al. 2005) and effective control-cross seed production (Bramlett 1993, Bramlett and Matthews 1983, Moody and Jett 1990), but more research must be done on pollen collection, handling, and testing to reduce the impact of poor pollen quality and close the gap between OP seed production and the mass production of control-cross seed. Considering that over 2.6 million pollination bags have been installed annually in the southeastern United States in the last two years, there is a great potential to increase control-cross seed yield, and thus forest productivity, through improved orchard management.

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This study was possible due to the contributions from full members of the North Carolina State University Cooperative Tree Improvement Program. Their orchard managers participated in this study for several years as they installed pollination bags, monitored strobili development, and harvested cones. The authors sincerely appreciate their time and effort in making this study possible. The authors would like to thank PBS International in the United Kingdom for supporting this research and providing the pollination bag prototypes tested in this study. The authors would also like to acknowledge the undergraduate and graduate students for their time and attention to detail in completing the cone analysis and seed extraction work, especially Rachel Powell, Haley Hollan, Paige Green, Jessica Maynor, Andrew Sims, and Serenia O'Berry.

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Conflict of Interest

None declared.

Supplementary Materials

Supplementary data are available at *Forest Science* online.

Supplemental Table S1. Description of pollination bag prototypes used in the study.

Supplemental Table S2. Number of cones analyzed by treatment and year of pollination in the cone analysis data set.

Supplemental Table S3. Model fit statistics (AIC) comparing fixed effects in the zero-inflated negative binomial model for seed yield with the preferred models in bold. For models excluding the open-pollinated (OP) treatment, the model with main effects for control-pollination efficiency (CPE) and number of strobili bagged (SB) in both the zero part and the count part of the model was preferred (model 8). When including the OP treatment, the preferred model had fixed effects for treatment (TRT), CPE, and SB in the zero part and the interaction of TRT and CPE with the main effect for SB in the count part (model 2).

Supplemental Figure S1. Scatterplot matrix of normally distributed cone analysis measurements and the control-pollination efficiency metric with Pearson correlation coefficient above the diagonal, histograms along the diagonal, and scatterplots with simple linear regression line below the diagonal. Cone length had a moderately high positive correlation with cone width and number of fertile scales.

Supplemental Figure S2. Scatterplot matrix of ovule counts by class and the control-pollination efficiency metric with Pearson correlation coefficient above the diagonal, histograms along the diagonal, and scatterplots with simple linear regression line below the diagonal. Filled seed and first-year aborts had a moderately strong negative relationship.

Supplemental Figure S3. Scatterplot matrix for counts of strobili pollinated, cones harvested, seed yield, and the control-pollination efficiency metric with Pearson correlation coefficient above the diagonal, histograms along the diagonal, and scatterplots with simple linear regression line below the diagonal. The seed yield had moderately strong relationships with number of strobili and the control-pollination efficiency.

Supplemental Figure S4. Hanging rootograms displaying the square root of observed and modeled frequencies of seed yield (for values up to three hundred) for four different models (Poisson, negative binomial, and their zero-inflated counterparts). The modeled frequencies are given by the red lines, and the observed frequencies (blue bars) are hung from the red line. Model fit is indicated by the blue bars touching the horizontal black line at zero. The zero inflated negative binomial performed the best.

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