Estimating Effect of *Megastigmus spermotrophus* (Hymenoptera: Torymidae) on Douglas-Fir Seed Production: The New Paradigm

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ABSTRACT In a pollen exclusion experiment performed on the cones of five Douglas-fir (*Pseudotsuga menziesii* [Mirbel] Franco) trees, the number of seeds infested by a seed chalcid, *Megastigmus spermotrophus* Wachtl, did not differ significantly between pollinated and unpollinated cones from the same tree. This finding led to a revision of the formula used to calculate *M. spermotrophus* effect on Douglas-fir seed production because the traditional formula, which is based on the assumption that only pollinated seeds are infested by these chalcids, exaggerates their effect. The relationship between the new formula and the traditional formula is nonlinear, varying with both pollination rate and infestation level. To assist other researchers in estimating the error in past chalcid studies, the discrepancies for a range of pollination rates were calculated. Past assessments were strongly biased only where pollination rates were <70% and chalcid attack rates were from 50 to 85% using the traditional formula. For low pollination rates, the discrepancy can exceed 50%. Evidence is presented to explain how the unfertilized female gametophyte, which is small and is normally resorbed in the absence of pollination, can support the development of chalcid larvae. In this study, there was a strong correlation (−0.93) between cone length and chalcid attack rate.

KEY WORDS *Megastigmus spermotrophus*, seed orchards, impact assessment

REFORESTATION OF DOUGLAS-FIR, *Pseudotsuga menziesii* [Mirbel] Franco, following harvests, wildfires, or pest epidemics depends on a reliable supply of high-quality seed. As a consequence, industry and government agencies have established conifer seed orchards for the production of such genetically improved seed, which has been valued at up to $2,000/kg.

Megastigmus spermotrophus Wachtl, a seed chalcid, is an important pest of Douglas-fir seed production both in the natural range of Douglas-fir in North America and in European countries where introduced Douglas-fir has become an important component of the timber economy. The seed chalcid has been reported to destroy 20% or more of Douglas-fir seed in the Pacific Northwest (Schowalter et al. 1985), and reported attack rates in European seed orchards often exceed 90% (Roques 1981). Seed chalcid impact assessments have previously been based on the assumption that chalcid larvae require pollinated ovules to mature successfully, because unpollinated ovules have only a small amount of tissue which is resorbed by the plant if pollination is unsuccessful (Allen & Owens 1972).

The formula traditionally used to calculate the chalcid attack rate (Lessmann 1974, Roques 1983), denoted by T, is based on the assumption that all chalcid-infested seeds would, in the absence of other cone and seed pests, have produced sound seeds:

$$T = \frac{Infested}{Infested + Sound}$$

where *infested* is the number of chalcid-infested seeds per cone, and *sound* is the number of remaining sound seeds per cone after chalcid attack.

If chalcids do not require pollinated seeds, then previous assessments of the effect of seed chalcid have clearly been inflated by an undetermined amount.

Results from earlier studies in northern California (N. R., unpublished data) and central France (A. R. & N. R., unpublished data) supported the hypothesis that chalcids do not require pollinated seeds to produce viable progeny, but chalcid populations were low in both cases, with infestation levels of 1–5% in both pollen-exposed and pollen-excluded cones. We concluded that a third study, with higher chalcid populations, was necessary to provide convinc-

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ing evidence that chalcid larvae in pollen-excluded cones did not result from accidental pollen contamination in those cones.

A second objective was to develop an accurate formula for assessing impact and to determine the potential discrepancies between this new formula and the traditional impact formula. The reason for calculating these potential discrepancies was to assess the magnitude of previous overestimates of the effect of chalcids; if these overestimates were small, those studies (Lessmann 1974, Hedlin et al. 1980, Roques 1981) retain some utility in ranking importance of seed chalcids compared with other pests of Douglas-fir seed production.

Finally, we wished to assess the correlation previously reported (Lessmann 1974, Rappaport & Roques 1991) between cone size and chalcid attack rate, using greater numbers of cones per tree and sampling them closer to the time of oviposition, which is when hosts are selected. Cone size is known to have a genetic component, so a strong correlation between cone size and chalcid attack might provide the basis for a genetic resistance program in seed orchard design.

Materials and Methods

The study was conducted at the USDA Forest Service Monmouth Breeding Orchard near Monmouth, OR. Four to 19 ramets each of 20 clones were grafted in this 2-ha orchard in 1973 and 1974; ramet height was maintained at a constant 5–6 m by topping. We selected five cone-bearing trees at random, each with at least 40 conebud clusters that had not yet flowered (budscales were still tightly appressed), then replicated each of the following four treatments 10 times on each tree (one cluster of seed cones represents one replicate): (1) exclude pollen, then expose cones to chalcids; (2) exclude both pollen and chalcids; (3) expose cones to pollen, then expose them to chalcids; (4) expose cones to pollen, then exclude chalcids.

Pollen exclusion was accomplished by enclosing cone clusters (two to eight cones) in pollination bags before budburst; female cones are receptive to pollination as soon as bracts emerge from the budscales (Ho 1980, Owens et al. 1981), so it was crucial to accomplish the bagging before that point. Male cone buds occur on terminal branches near female cones; to avoid self-pollination within the pollen exclosure bags, we identified male cones using the criteria of Allen & Owens (1972) and removed all of them by hand. To reduce the possibility of contamination by errant pollen grains, we rinsed female buds with water before bagging them. This served the dual purpose of washing off any pollen grains that might have been present and of forcing their premature germination.

Table 1. Mean number (SD) of *M. spermotrophus*-infested seeds per cone

Tree no.	Chalcid-exposed cones		Chalcid-excluded cones	
	Without pollen	With pollen	Without pollen	With pollen
1	20.9 (4.2)	22.9 (7.5)	0 (0)	0 (0)
2	15.0 (4.3)	8.4 (3.9)	0 (0)	0 (0)
3	14.8 (6.7)	12.1 (6.2)	0 (0)	0 (0)
4	4.9 (3.1)	3.3 (2.9)	0 (0)	0 (0)
5	6.3 (3.8)	5.0 (2.5)	0 (0)	0 (0)
Avg	12.4 (7.6)	10.3 (8.6)	0 (0)	0 (0)

Chalcid exclusion was accomplished by enclosing cones in pollination bags during the seed chalcid oviposition period (from mid-April to mid-July). In mid-July, one cone was randomly chosen from each treatment and was measured and dissected scale by scale to assess chalcid presence before the formation of galls of *Contarinia oregonensis* Foote (Diptera: Cecidomyiidae), which might have interfered with our assessments. Voucher specimens have been deposited at the USDA, Forest Service, Pacific Southwest Research Station, Albany, CA.

Results and Discussion

Development of Chalcids in Unpollinated Seeds. Pollen exclusion and chalcid exclusion treatments were successful: no sound seeds occurred in the pollen exclusion treatments, and no chalcids were found in the chalcid exclusion treatments (Tables 1 and 2). Numbers of chalcids per cone for pollinated and unpollinated cones from the same tree were not significantly different (Wilcoxon signed rank test, $\alpha = 0.05$) (Snedecor & Cochran 1967) (Table 1). These results indicate that chalcids apparently do not distinguish between pollinated and unpollinated seeds, a finding in agreement with recent results reported by Niwa & Overhulser (1992).

At the time of fertilization, the female gametophyte does not appear to contain enough nutrient to support the development of a chalcid larva; if fertilization does not occur, the clear, watery female gametophyte is resorbed (Owens et al. 1991). If pollination and successful fertilization do ensue, however, the female gametophyte, un-

Table 2. Mean number (SD) of sound, uninfested seeds per cone

Tree no.	Chalcid-exposed cones		Chalcid-excluded cones	
	Without pollen	With pollen	Without pollen	With pollen
1	0 (0)	6.1 (4.4)	0 (0)	21.4 (5.9)
2	0 (0)	2.4 (3.3)	0 (0)	9.4 (6.7)
3	0 (0)	1.1 (1.4)	0 (0)	14.5 (3.8)
4	0 (0)	14.1 (7.9)	0 (0)	20.8 (9.1)
5	0 (0)	24.2 (13)	0 (0)	33.5 (10.6)
Avg	0 (0)	9.6 (11)	0 (0)	19.9 (11.2)

der hormonal stimulation from the developing embryo, develops within 3 mo to a large, opaque, solid mass containing sufficient nutrient to sustain the plant embryo through germination.

Owens et al. (1981) use the criterion of normal development of the nucellus as an index of successful embryogenesis; the nucellus, a gray membrane that surrounds the female gametophyte, is clearly visible through the immature seed coat from about 1 July to 1 August. All chalcid-infested seeds from pollen-excluded cones in our study showed normal nucellar development, which was unexpected because embryogenesis is exceedingly rare in the absence of pollination (Allen 1942, Orr-Ewing 1957). This phenomenon occurred only in pollen-excluded seeds that had been subjected to chalcid attack; all pollen-excluded megagametophytes that were protected from chalcid attack aborted and were completely resorbed. We found no embryos in the chalcid-infested, pollen-excluded seeds (chalcid larvae had already consumed the central seed tissues), but many of these seeds still contained female gametophyte tissue that appeared to be normal. Either oviposition or some factor associated with the larvae, then, must stimulate continued development of the female gametophyte in unpollinated seeds. This finding explains how unfertilized seeds, which normally cease to develop, can support the development of chalcid larvae.

Revised Formula for Estimating Effect of Chalcids. We developed a new formula for calculation of chalcid attack rate, denoted by N, that accounts for the fact that some chalcid-infested seeds might not have produced sound seeds in the absence of chalcids:

$$N = \frac{Pollinated - Sound}{Pollinated}$$

where *pollinated* is the number of pollinated seeds per cone, and *sound* is the number of remaining sound seeds per cone after chalcid attack.

The number of pollinated seeds per cone is estimated by counting the number of sound seeds in chalcid-excluded cones of the experiment. This method requires the exclusion of chalcids from a sample of cones; the minimum number of replicates required depends on chalcid population variability and must be calculated for each site and for the desired statistical power. It also requires that other pest species in the complex—*C. oregonensis, Barbara colfaxiana* (Kearfott) (Lepidoptera: Olethreutidae), *Dioryctria abietivorella* Grote (Lepidoptera: Pyralidae), and *Leptoglossus occidentalis* Heidemann (Hemiptera: Coreidae)—be excluded from that subset of cones to derive an accurate assessment of the production of potentially sound seed. Because chalcid exclusion from a subset of cones is necessary for an assessment of impact, precise retroactive assessments are not possible. Thus, many previous chalcid impact studies clearly have inflated impact values. To the extent that chalcids mask the occurrence of unfertilized seeds, the impact of other cone and seed insects has also been inflated.

Relationship Between New and Traditional Impact Formulas. The impact of chalcids, as estimated by the traditional formula, will be biased depending on the rate of pollination of seeds and the rate of infestation. The formula below shows the mathematical relationship, which is nonlinear, between the two chalcid attack rates T and N. It is assumed that pollinated seeds are just as likely to be attacked as unpollinated seeds, an assumption that we are currently testing:

$$T = \frac{N}{P + N(1 - P)} \tag{1}$$

where P is the pollination rate per cone, N is the chalcid attack rate using the new impact formula, and T is the chalcid attack rate using the traditional impact formula.

If the pollination rate is 100%, then $T = N$, and the two formulas give identical results. If $P < 1.0$, as is normally the case, then $T > N$. In other words, for all real-world ranges of pollination, the traditional formula produces an inflated estimate of chalcid damage. The degree of discrepancy between the traditional and new attack rate formulas can be assessed by the formula for their difference as a function of the traditional rate and the pollination rate, given in equation 3 below: we obtain N from equation 1 above:

$$N = \frac{TP}{1 - T(1 - P)}, \tag{2}$$

$$\text{then} \quad T - N = T - \frac{TP}{1 - T(1 - P)}$$

$$= \frac{T - T^2(1 - P) - TP}{1 - T(1 - P)}, \tag{3}$$

$$\text{so} \quad T - N = \frac{(1 - P)(T - T^2)}{1 - T(1 - P)}.$$

Figure 1 indicates that past impact assessments were strongly biased only where pollination rates were <70% and chalcid infestations were from 50–85% using the traditional formula. For a pollination rate of 10% and chalcid infestation rate (T) of 75%, however, the discrepancy exceeds 50%. According to previous impact studies, chalcid infestation rates are characteristically much higher in Europe than in North America; North American infestation rates are usually <20% (Hedlin et al. 1980, Schowalter et al. 1985), whereas rates of 30–90% are common in Europe (Roques 1983). Pollination rates are im-

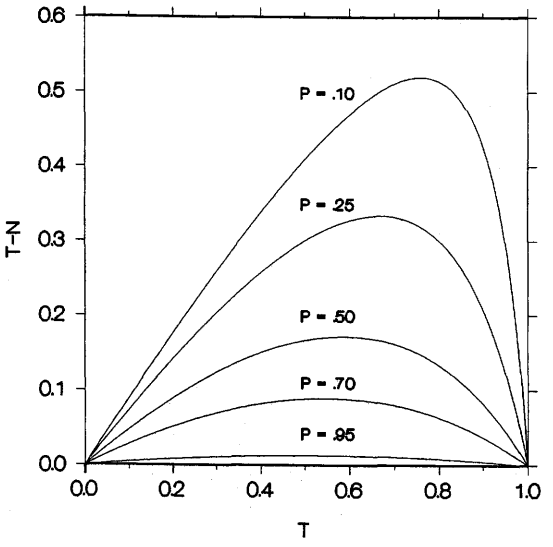

Fig. 1. Discrepancies between the new *(N)* and traditional *(T)* formulas that measure impact of *M. spermotrophus* for five different pollination *(P)* rates and all possible values of *T*.

possible to sample after the fact, but a 2-yr study by Ho (1980) in Oregon seed orchards reports that an average of 70% of Douglas-fir ovules contained one or more germinated pollen grains, a rate of pollination that would be consonant with an error rate $(T-N)$ of 9% for a value of T of ≈50%. Studies conducted in British Columbia report average rates of seed efficiency from 39–85% (seed efficiency is the percentage of normal, filled seeds out of the total of potential seeds per cone; i.e., $SE = 100 \times P$, where SE is seed efficiency) (McAuley 1990, Owens et al. 1991). Seed efficiency has been shown to vary with reproductive phenology in Douglas-fir (El-Kassaby et al. 1984): in 1981, trees flowering early and late had seed efficiencies of 33% and 26%, respectively, whereas those with intermediate flowering phenologies had seed efficiencies of 50%. In 1982, the seed efficiencies for the same trees were 62% (early), 57% (late), and 85% (intermediate). When assessing chalcid impact, then, seed efficiency must be sampled by excluding all seed and cone insect species from a subset of cones on each tree.

Empirical Comparison of Old and New Formulas. The data from this study were used to estimate the two chalcid attack rates (T and N) and their difference ($T - N$) per tree. In addition, with the estimates of T and N, an estimated pollination rate can be obtained with equation 4 below, which is derived as follows:

from equation 1 above, $$T = \frac{N}{P + N(1 - P)},$$

so $$N = TP + TN - TNP,$$

Table 3. Estimated new and old chalcid infestation rates, their differences, and pollination rates in percentages

Parameter	Tree				
	1	2	3	4	5
New formula	72	75	92	32	28
Old formula	79	78	92	19	17
Difference	7	3	0	−13	−11
Pollination rate	67	84	100	—	—

and therefore $$N - TN = P(T - TN),$$

so $$P = \frac{N - TN}{T - TN} = \frac{N(1 - T)}{T(1 - N)}$$

and if $\hat{N}$ is an estimator of N and $\hat{T}$ is an estimator of T, then one can estimate $\hat{P}$ (estimated rate of pollination) as

$$\hat{P} = \frac{\hat{N}(1 - \hat{T})}{\hat{T}(1 - \hat{N})}. \quad (4)$$

Table 3 shows the two estimated chalcid attack rates and the estimated differences calculated by averaging the total number of seeds (sound or chalcid-infested) by the number of cone clusters (10 clusters, one cone per cluster). If the same estimation is made per cluster, then the standard error of the estimates can be calculated. The differences $(T - N)$ for two of the trees were negative because the new method of determining chalcid impact is a stochastic process; negative differences will be less likely using larger sample sizes.

Correlations Between Cone Size and Attack Rate. Previous studies (Lessmann 1974, Rappaport & Roques 1991) have shown a correlation between cone size and rate of attack by the seed chalcid, smaller cones often being more heavily attacked than larger cones. The refined impact formula described above was used to test the correlation between cone size and attack rate at Monmouth in 1991. Our results show a closer correlation than previously seen (Lessmann 1974, Rappaport & Roques 1991), with a correlation coefficient of $r = -0.93$ between cone length and chalcid attack rate using the new formula (Fig. 2). A possible explanation for this high correlation is that cones were sampled and measured in mid-July, just a few weeks after chalcid oviposition. Cones sampled early in the season have not yet been influenced by other factors, such as attack by other cone and seed insects or differential growth resulting from nutritional differences, that might affect ultimate cone size.

Our results show the importance of a full understanding of insect–host interactions in assessing pest impact. The mathematical relationships presented provide a means of estimating the error in past chalcid impact calculations if the researcher can reconstruct the pollination rate of

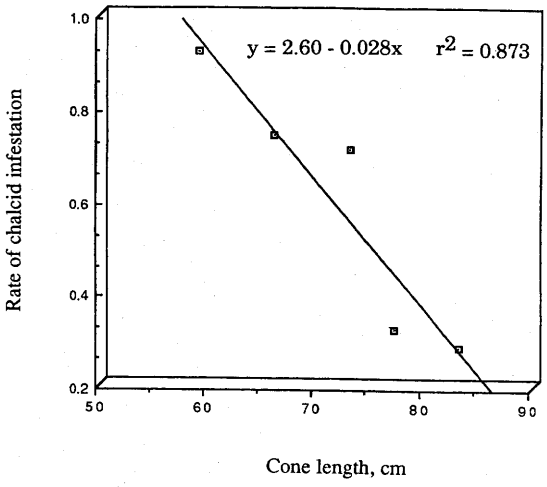

Fig. 2. Relationship between mean cone length (cm) and mean numbers of seeds destroyed per cone by *M. spermotrophus* for five trees, Monmouth Breeding Orchard, 1991.

those past studies, but future impact studies must be designed to account for the new information about chalcid development in unpollinated seeds. Our results demonstrate that, for pollination rates of >70%, the traditional formula for calculating chalcid impact is no higher than 10% of the actual value. For low pollination rates and rates of chalcid infestation that are intermediate to high, the discrepancies are substantial.

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