ABSTRACT
Fungi of the genus Mycosphaerella is one of the major leaf disease of Eucalyptus globulus worldwide. The main species that infect E. globulus in southern Australia are M. cryptica and M. nubilosa. M. nubilosa is mainly confined to the juvenile foliage whereas M. cryptica may occur on both foliage types. Mycosphaerella damage to E. globulus plantations can be severe and the risk of disease damage is one of the main reasons for the shift towards planting the more resistant E. nitens in northern Tasmania. We examined the quantitative genetic variation in susceptibility to infection by M. nubilosa in a genetically diverse population of E. globulus families growing in a field trial in north-west Tasmania. The trees were two years old and still entirely in the juvenile foliage stage when a heavy epidemic of M. nubilosa occurred. Disease incidence was uniform across the trial and the mean leaf area damage (severity assessed as % of necrotic of lost leaves on whole tree basis) was very high at 34%. Significant genetic variation for susceptibility was detected with a narrow-sense heritability of disease severity of 0.6 being the highest yet reported for a Mycosphaerella disease of eucalypts. We followed the effects of this disease outbreak on growth up to age 7 years and found that M. nubilosa damage had a significantly deleterious impact on tree growth at both the phenotypic and genetic level. At age 7, the top 10% of families had a mean DBH 20.8% greater than the trial mean. Approximately half of this gain would have been achieved by early selection for disease resistance (9.1%) or height (11.0%) at age 2, with a time advantage of 5 years. This is similarly the case for selection of above average families. It is likely such gains would be reduced in homogenous plantings of resistant genotypes, or if genotype x environment interactions are significant. Nevertheless, a large component of this gain is likely to be due to disease resistance per se, and collection of seed from resistant seed orchard parents offers the potential for rapid gains in productivity in plantations at risk of disease damage.
main reasons for the shift towards planting the more resistant *E. nitens* in northern Tasmania. We examined the quantitative genetic variation in susceptibility to infection by *M. nubilosa* in a genetically diverse population of *E. globulus* families growing in a field trial in north-west Tasmania. The trees were two years old and still entirely in the juvenile foliage stage when a heavy epidemic of *M. nubilosa* occurred. Disease incidence was uniform across the trial and the mean leaf area damage (severity assessed as % of necrotic of lost leaves on whole tree basis) was very high at 34%. Significant genetic variation for susceptibility was detected with a narrow-sense heritability of 0.6 being the highest yet reported for a *Mycosphaerella* disease of eucalypts. We followed the effects of this disease outbreak on growth up to age 7 years and found that *M. nubilosa* damage had a significantly deleterious impact on tree growth at both the phenotypic and genetic level. At age 7, the top 10% of families had a mean DBH 20.8% greater than the trial mean. Approximately half of this gain would have been achieved by early selection for disease resistance (9.1%) or height (11.0%) at age 2, with a time advantage of 5 years. This is similarly the case for selection of above average families. It is likely such gains would be reduced in homogenous plantings of resistant genotypes, or if genotype x environment interactions are significant. Nevertheless, a large component of this gain is likely to be due to disease resistance per se, and collection of seed from resistant seed orchard parents offers the potential for rapid gains in productivity in plantations at risk of disease.

**INTRODUCTION**

Economic returns from plantations are sensitive to delays in harvest age caused by slower than projected growth (Candy and Gerrand 1997). Pests that impair growth by parasitising or browsing on *E. globulus* foliage are thus receiving increased attention. In addition, the control of pests that cause early senescence of branches, which cannot be pruned before dying, may also be important for wood quality (Montagu et al. 2003). Genetic variation for susceptibility to several pests has been demonstrated in *E. globulus* (Lundquist and Purnell 1987; Carnegie et al. 1994; Dungey et al. 1997; Dutkowski and Potts 1999; Table 1). The number of progeny per OP family ranged from 11 to 42. The grand-mean number of progeny per race ranged from 48 to 207 (Dutkowski and Potts 1999; Table 1). The trial contained 12 replicates, with 72 individuals per replicate. The whole trial was surrounded by a buffer row and each replicate contained one to three individuals from each family arranged at random.

**Mycosphaerella** species cause leaf diseases on eucalypts worldwide (Park and Keane 1984; Park 1988b; Carnegie and Keane 1994; Crous 1998; Mohammed et al. 2003). The fungal species that have the most impact on *E. globulus* in southern Australia are *Mycosphaerella cryptica* (Cke) Hansf. and *M. nubilosa* (Cke) Hansf. (Carnegie et al. 1998; Milgate et al. 2001). There is evidence of genetic variation in susceptibility to these pests at the provenance and family level in *E. globulus* (Carnegie et al. 1994; Dungey et al. 1997). *Mycosphaerella nubilosa* has been found on seven *Eucalyptus* species in Australia and New Zealand (Crous 1998). Once deposited onto a leaf, ascospores are able to remain viable and infective for up to seven days (Park 1988a). According to Park and Keane (1982b) and Park (1988a), *M. nubilosa* does not appear to be able to infect leaves by direct penetration of the cuticle and does not normally infect adult foliage. Leaf infection appears to occur when ascospores germinating in the surface produce germ tubes, which enter the leaf via the stomates (Park and Keane 1982b). Once this pathogen enters the stomatal cavity it undergoes a period of ramification into intercellular spaces. This occurs during an incubation period of 3 to 6 weeks before lesions becomes visible (Park 1988a). At this point pseudothecia become visible in the stomatal cavities. The work presented here was initiated when a damaging epidemic of *M. nubilosa* occurred in a genetic field trial of *E. globulus* in north-west Tasmania. This epidemic allowed us to study the quantitative genetic control of susceptibility to *M. nubilosa* damage and the effect of the disease on *E. globulus* growth.

**MATERIALS AND METHODS**

**Trial site and genetic material.** Susceptibility to *Mycosphaerella* leaf blotch was studied in a Gunns Ltd. *E. globulus* field trial near Ridgley, in north-western Tasmania (Lat. 41°15′ Long 145°48′). Prior to the establishment of the trial the land was pasture. The trial was established in November 1996 with 864 trees originating from 53 open-pollinated (OP) families collected in a seed orchard at Woolnorth owned by Gunns Ltd. (Volker et al. 1990). The number of progeny per OP family ranged from 11 to 42. The grand-parents of the OP families were from seven native stand races of *E. globulus* and the number of progeny per race ranged from 48 to 207 (Dutkowski and Potts 1999; Table 1). The trial contained 12 replicates, with 72 individuals per replicate. The whole trial was surrounded by a buffer row and each replicate contained one to three individuals from each family arranged at random.

**Disease assessment.** In September 1998, a severe epidemic of *Mycosphaerella* leaf disease developed in the trial. At this time, most trees were entirely in their juvenile phase. An inspection of the trial in May 1997 had found only very low incidence of disease. In October 1998, to determine the species present, ten typical large blighting lesions were collected from different trees in the trial that represented the range of necrotic leaf symptoms expected for *Mycosphaerella*. These were then examined microscopically measuring 30 ascospores, pseudothecia, asci, germinating ascospores and single ascospore cultures (Park and Keane,
and Dutkowski (REML) mixed model following Borralho (1995) individual tree, restricted maximum likelihood replicate effects were estimated using an components due to additive genetic and 

the pedigree file was assigned to a single Woolnorth seed orchard, the pollen parents in families which were derived from the trees in the case of the generation of open-pollinated expected based on geographic proximity. In the same genetic group as the female, as would be unknown, they were assumed to come from the pollenated families grown at Woolnorth were derived. As the pollen parents of these open-pollinated from which race the wild grandparents were pedigreed included the genetic group indicating common grandparents. In the present case, the some of the open-pollinated families sharing derived from a multi-generation pedigree, with trees in the trial were directly (Gilmour matrix from which additive effects are estimated pedigree information to construct a relationship 2001). This approach was necessary as the trees in the trial were four parental generation of Eucalyptus globulus genetic material in the trial, with number of grand-parents, parents (female parent of OP families) and progeny in each genetic group, together with their least square mean for Mycosphaerella nubilosa % Severity. Means that have different letter(s) differ by two standard errors

<table>
<thead>
<tr>
<th>Genetic groups</th>
<th>No. of grand-parents</th>
<th>No. of parents</th>
<th>No. of progeny</th>
<th>% Severity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Southern Tasmania</td>
<td>4</td>
<td>5</td>
<td>58</td>
<td>26.5*</td>
</tr>
<tr>
<td>Western Tasmania</td>
<td>7</td>
<td>15</td>
<td>207</td>
<td>41.4**</td>
</tr>
<tr>
<td>King Island</td>
<td>3</td>
<td>11</td>
<td>196</td>
<td>25.8*</td>
</tr>
<tr>
<td>Recherche</td>
<td>2</td>
<td>5</td>
<td>58</td>
<td>17.4*</td>
</tr>
<tr>
<td>Furneaux</td>
<td>2</td>
<td>7</td>
<td>138</td>
<td>52.2**</td>
</tr>
<tr>
<td>Western Otways</td>
<td>3</td>
<td>7</td>
<td>157</td>
<td>42.6**</td>
</tr>
<tr>
<td>NE Tasmania</td>
<td>2</td>
<td>3</td>
<td>48</td>
<td>29.4**</td>
</tr>
</tbody>
</table>


development of tabulation

Table 1. Origin of Eucalyptus globulus genetic material in the trial, with number of grand-parents, parents (female parent of OP families) and progeny in each genetic group, together with their least square mean for Mycosphaerella nubilosa % Severity. Means that have different letter(s) differ by two standard errors

The disease severity (% Severity) of each plant was recorded in October 1998. The percentage of necrotic leaves on a whole tree basis was scored on a ten point scale (1=0-3%, 2=4-6%, 3=7-12%, 4=13-17%, 5=18-25%, 6=26-38%, 7=39-50%, 8=51-63%, 9=64-75%, 10=76-100%). Tree height was measured in April 1997 (HEIGHT1) prior to infection, during the infection (December 1998, HEIGHT2) and after the infection in August 2000 (HEIGHT4). Diameter at breast height was measured in August 2000 (DBH4) and June 2003 (DBH7).

Estimation of genetic components and correlations. In order to separate genetic and environmental effects on E. globulus susceptibility to M. nubilosa, variance components due to additive genetic and replicate effects were estimated using an individual tree, restricted maximum likelihood (REML) mixed model following Borrallho (1995) and Dutkowski et al. (2002). These analyses were implemented using ASReml (Gilmour et al. 2001). The individual tree approach uses known pedigree information to construct a relationship matrix from which additive effects are estimated directly (Gilmour et al. 2001). This approach was necessary as the trees in the trial were derived from a multi-generation pedigree, with some of the open-pollinated families sharing common grandparents. In the present case, the pedigree included the genetic group indicating from which race the wild grandparents were derived. As the pollen parents of these open-pollinated families grown at Woolnorth were unknown, they were assumed to come from the same genetic group as the female, as would be expected based on geographic proximity. In the case of the generation of open-pollinated families which were derived from the trees in the Woolnorth seed orchard, the pollen parents in the pedigree file was assigned to a single genetic group representing the pollen environment of the Woolnorth seed orchard. The model fitted included random terms for the additive genetic ($\sigma^2_a$), replicate ($\sigma^2_r$) and the genetic group effect estimated as a fixed term. Overall breeding values (and standard errors) for the grandparents, parents and progeny were calculated directly by the program and included the additive genetic effect plus the genetic group effect. Variance components used to estimate heritabilities were derived from univariate analyses. These values were then used as starting values for bivariate analyses using the same model to estimate genetic and environmental (replicate) correlations. Phenotypic correlations were estimated using PROC CORR of SAS (Version 8). The narrow-sense heritabilities ($h^2$) were calculated as: $h^2 = \frac{\sigma^2_a}{\sigma^2_a + \sigma^2_e}$ where: $\sigma^2_a$ is the additive genetic variance within genetic groups and $\sigma^2_e$ is the error variance component. Standard errors of estimates were calculated by ASReml from the average information matrix, using a standard truncated Taylor series approximation (Gilmour et al. 2001). Heritabilities were adjusted to assume a coefficient of relatedness between open-pollinated sibs of 0.4. The significance of the heritability estimates were obtained using Z-tests, whereas the significance of the deviation of the correlation estimates from zero were based on likelihood ratio tests (LRT). These genetic correlations reflect only additive genetic associations within genetic groups while the correlations between replicate reflect only associations at a meso-environmental scale and are called environmental correlations [see Jordan et al. (2000)]. To predict tree height and diameter at varying levels of the disease, regression analysis was performed on phenotypic measurements of HEIGHT4 and DBH7 on % Severity using the Proc REG procedure of SAS.
RESULTS

Disease assessment. The amount of leaf damage caused by *M. nubilosa* in this trial was high with a mean of 34% of leaf area necrotic (% Severity averaged across all trees in the trial). However, at the individual tree level, the damage was extremely variable with the foliage of some trees almost completely damaged or shed while other trees were virtually undamaged (Fig. 1).

Heritability and genetic variation between genetic groups. There were significant differences between the genetic groups in their susceptibility to *M. nubilosa*. The nine grandparents from Recherche Bay, King Island and Southern Tasmania were significantly more resistant than the two grandparents from Furneaux (Table 1). There was also significant additive genetic variation for % Severity within genetic groups and the narrow-sense heritability was high and significant ($h^2_{op} = 0.60$). The narrow-sense heritability of first year growth was very low (HEIGHT1 - $h^2_{op} = 0.08$) and not significant. However, it increased with age to become highly significant by year 2 (HEIGHT2 - $h^2_{op} = 0.33$ Table 2). DBH also had a moderately high narrow-sense heritability at age 4 (DBH4 - $h^2_{op} = 0.44$), which also increased with age (DBH7 - $h^2_{op} = 0.52$).

Relationship between disease severity and growth. The phenotypic correlation between growth and % Severity was initially positive (HEIGHT1) with the faster growing plants being more susceptible to the disease (Table 2). Prior to infection there was no significant genetic variation in growth in the trial and hence no significant genetic correlation between growth and % Severity. The disease spread rapidly before the third growing season and significant negative genetic correlations between % Severity and plant height 25 months after planting when plants were in the middle of their third growing season. This meant that the plants that were genetically more susceptible grew less than those that were more resistant. By the fourth year, the deleterious effects of the disease were evident in the phenotypic and genotypic correlations with both height and diameter (Table 2). At this stage, every 1% increase in % Severity resulted in a 1.1 cm reduction in height ($r^2 = 5.1%$; $P < 0.001$) and a 0.22 mm reduction in diameter at breast height ($r^2 = 5.5%$; $P < 0.001$) at the phenotypic level. At year 7, a 1% increase in % Severity resulted in a 0.57 mm reduction in diameter. This result contrasts with the stronger genetic association between % Severity and growth, where for example at year 7, a 1% reduction in % Severity resulted in a 0.84 mm increase in diameter growth ($r^2 = 33%$; $P < 0.001$). Selection of undamaged families can lead to significant growth and economic advantages if deployed on similar disease susceptible sites. At age 7, the top 10% of families had a mean DBH 20.8% greater than the trial mean (Table 3). Approximately half of this gain would have been achieved by early selection for disease resistance (9.1%) or height (11.0) 2 years after planting, with a time advantage of 5 years. This is similarly the case for selection of above average families.

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**Table 1.** Variation in disease %Severity on individual trees in the *E. globulus* trial near Ridgley, in northwestern Tasmania. The replicate boundaries are shown as black lines and those replicates on the left experienced the least damage.

![Figure 1.](image)
in previous studies.

The resistance level found in the same provenance between the localities used in our study with small to allow comparison of resistance level size (i.e. number of grand-parents) was too resistance. However, the provenance sample represented a fair assessment of the genetic material in the trial ensured that this trial genetic groups. The broad base of genetic diversity in Eucalyptus globulus was assessed for Mycosphaerella damage (% Sev) in Carnegie and Ades 2002 (Rainoso 1992, Carnegie 2000). In our trial, 19% of trees had more than 50% leaf damage (% Severity). This damage appeared to be caused by a single Mycosphaerella species, M. nubilosa. Mycosphaerella nubilosa is confined to the juvenile foliage of E. globulus according to Carnegie and Ades 2002. In other studies leaf damage has involved more than one Mycosphaerella species (e.g. M. cryptica and M. nubilosa in both Dungey et al. 1997; Carnegie and Ades 2002).

In this trial, the estimation of the level of additive genetic variation within the genetic groups was complicated by the fact that many of the progeny had parents that were related through sharing a common grand-parent. It was therefore necessary to use an individual tree model. Such relatedness issues will be an increasingly common problem as tree breeding programs shift to advanced generations and seed orchards are progeny tested. Our heritability estimate of M. nubilosa severity ($h^2_{op} = 0.6 \pm 0.12$) was higher than any previously recorded for either juvenile ($h^2 = 0.12$ - Dungey et al. 1997; $h^2 = 0.31-0.32$ - Rainoso 1992) or adult foliage ($h^2 = 0.23$ - Dungey et al. 1997; $h^2 = 0.17$ - Carnegie 2000) of E. globulus. Dungey et al. (1997) estimated heritabilities from both OP and controlled pollinated progenies and showed that they were comparable to one another. This result gave us confidence that our open-pollinated heritability for % Severity was accurate and must be reflecting true additive genetic variation in E. globulus.

There are several reasons, which could explain why genetic variation in susceptibility to the Mycosphaerella disease was so well expressed in our trial (i.e. high heritability). Firstly, the absence of variation in disease incidence traits (data not shown) showed that nearly all the trees in the trial were exposed to infection and thus the possibility that resistant plants escaped infection was low. Such 'escape' is often believed to be the cause of the low heritability of some pest traits (e.g. marsupial browsing $h^2_{op}=0.13$ - O’Reilly-Wapstra et al. 2002). Secondly, the severity of the disease in our trial was the highest yet studied and high disease severity may be more optimal for the expression of genetic differences. A study of resistance to fusiform rust infection in 171 trials of loblolly pine in the USA showed that heritability estimates increased linearly with increasing average rust infection in the trials (Dieters et al. 1996). Thirdly, only one pathogen was found to cause the bulk of the foliage damage in our study, whereas damage in at least some of the other studies has involved at least two different species of Mycosphaerella (e.g. M. nubilosa and M. cryptica – Rainoso 1992; Dungey et al. 1997; Carnegie and Ades 2002). Finally, the replicate design in this trial was very efficient in removing environmental variation in disease severity as

### Table 3. The percentage improvement in diameter at age 7 (DBH7) over the trial mean following selection of (1) the top 10% families or (2) above average families for height (HEIGHT), diameter (DBH) or Mycosphaerella damage (% Sev) scored in year 2. The highest gains are achieved by selecting at year 7 using strategy 1, but half of these gains could have been made 5 years earlier by selecting at year 2.

<table>
<thead>
<tr>
<th>Selection strategy</th>
<th>SEV</th>
<th>HEIGHT1</th>
<th>HEIGHT2</th>
<th>HEIGHT4</th>
<th>DBH4</th>
<th>DBH7</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Top 10% of families</td>
<td>1.4</td>
<td>9.1</td>
<td>11.0</td>
<td>16.4</td>
<td>17.8</td>
<td>20.8</td>
</tr>
<tr>
<td>2. Above average families</td>
<td>3.1</td>
<td>6.3</td>
<td>6.2</td>
<td>7.2</td>
<td>7.8</td>
<td>9.5</td>
</tr>
</tbody>
</table>

**DISCUSSION**

The severity of Mycosphaerella leaf disease in the present trial was the highest among reported studies of Mycosphaerella genetics in Eucalyptus globulus. For example, the mean % Severity of 34% was greater than even the maximum individual tree damage levels previously reported of 8% to 27% (Rainoso 1992; Carnegie et al. 1994; Dungey et al. 1997; Carnegie 2000). In our trial, 19% of trees had more than 50% leaf damage (% Severity). This damage appeared to be caused by a single Mycosphaerella species, M. nubilosa. Mycosphaerella nubilosa is confined to the juvenile foliage of E. globulus according to Carnegie and Ades 2002. In other studies leaf damage has involved more than one Mycosphaerella species (e.g. M. cryptica and M. nubilosa in both Dungey et al. 1997; Carnegie and Ades 2002).

Genetic variation in our trial occurred at two levels, between the genetic groups from which the grand-parents were derived and within these groups. The differences between the genetic groups are fixed genetic differences derived from sampling trees from different provenances throughout the geographic range of E. globulus. Initially a larger number of trees were sampled from the natural population and their open-pollinated progenies planted in a trial at Woolnorth (Volker et al. 1990). However, when this trial was converted to a seed orchard, many families and individuals were culled. In addition, not all of the remaining trees in the orchard were sampled to establish the progeny trial that we assessed for Mycosphaerella. Provenance differences in Mycosphaerella resistance have been reported in trials derived from wild collections of E. globulus (Rainoso 1992; Carnegie et al. 1994) and such variation could account for the genetic differences between our genetic groups. The broad base of genetic material in the trial ensured that this trial represented a fair assessment of the genetic diversity in E. globulus for M. nubilosa resistance. However, the provenance sample size (i.e. number of grand-parents) was too small to allow comparison of resistance level between the localities used in our study with resistance level found in the same provenance in previous studies.

As in Carnegie and Ades 2002, the genetic differences in disease incidence traits (data not shown) showed that nearly all the trees in the trial were exposed to infection and thus the possibility that resistant plants escaped infection was low. Such 'escape' is often believed to be the cause of the low heritability of some pest traits (e.g. marsupial browsing $h^2_{op}=0.13$ - O’Reilly-Wapstra et al. 2002). Secondly, the severity of the disease in our trial was the highest yet studied and high disease severity may be more optimal for the expression of genetic differences. A study of resistance to fusiform rust infection in 171 trials of loblolly pine in the USA showed that heritability estimates increased linearly with increasing average rust infection in the trials (Dieters et al. 1996). Thirdly, only one pathogen was found to cause the bulk of the foliage damage in our study, whereas damage in at least some of the other studies has involved at least two different species of Mycosphaerella (e.g. M. nubilosa and M. cryptica – Rainoso 1992; Dungey et al. 1997; Carnegie and Ades 2002). Finally, the replicate design in this trial was very efficient in removing environmental variation in disease severity as
judged from the highly significant replicate effect (result not shown).

**Impact on growth.** The heritability of both height and diameter at four years in our trial were the highest yet reported in *E. globulus* and are nearly double the average values reported in a review of genetic parameter estimates in this species by Lopez et al. (2002). This was no doubt due to the good expression of genetic variation for disease resistance in our trial, coupled with the strong, deleterious impact of the disease on growth. Productivity was clearly adversely affected by the severity of *Mycosphaerella* disease at both the genetic and phenotypic level. The phenotypic regressions showed that for every percentage increase in foliage loss due to disease in the year of the epidemic there was a 1.22cm loss of potential height gain at four years. This means that a phenotypic decrease in disease severity score of 10% would result in a 3.1% increase in growth. However, in other cases, this effect of disease on growth may be even greater. Carnegie and Ades (2002) have reported that in a fungicide trial, less than 10% of leaf area damage caused by *Mycosphaerella* decreased height increment by up to 13% over 17 months of *E. globulus*. A likely explanation for the differences between the two studies was the use of fungicides to exclude disease while in our trial no tree completely escaped the disease. At the genetic level, the relationship between growth and disease severity was even stronger, clearly indicating that selection for improved disease resistance will indirectly result in improved growth on sites affected by *Mycosphaerella*. From another perspective, selection of trees with high breeding value for growth on sites exposed to high disease risk. In this case, we recommend progeny testing deployment populations (from seed orchard, control or mass pollinated families, or clones) on disease prone sites to allow ranking for disease resistance and later age growth in the presence of disease.

**Exploiting disease resistance.** There is clearly a large amount of genetic variation in the *E. globulus* gene pool for resistance to *Mycosphaerella* leaf disease. Given a good disease outbreak this genetic variation can be strongly expressed allowing effective selection of resistant genotypes. Exploitation of this variation can take place in the breeding or deployment stage. Exploitation at the deployment stage is probably a favored option, as not all of the plantation estate has a high disease risk. In this case, we recommend progeny testing deployment populations (from seed orchard, control or mass pollinated families, or clones) on disease prone sites to allow ranking for disease resistance and later disease outbreak this genetic variation can be escaped the disease. At the genetic level, the relationship between growth and disease severity was even stronger, clearly indicating that selection for improved disease resistance will indirectly result in improved growth on sites affected by *Mycosphaerella*. From another perspective, selection of trees with high breeding value for growth on sites exposed to high disease risk. In this case, we recommend progeny testing deployment populations (from seed orchard, control or mass pollinated families, or clones) on disease prone sites to allow ranking for disease resistance and later age growth in the presence of disease.

**REFERENCES**


