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Seed vigour testing for predicting field seedling emergence in *Fagus sylvatica* L.

Abstract: A vigour test that can predict the field emergence percentage of tree seeds accurately has long been a wish from growers. A new test method, the Critical Root Length (CRL) vigour test, was developed for beech seeds on the basis of the length of primary roots, germinated seeds can produce during a specified test. Pretreated, imbibed seeds were germinated in a vertically positioned moist paper roll during 20 days at 15°C in 12 hour light daily. Root length of normally germinated seedlings was recorded and correlated with field emergence percentage to obtain a critical root length for the ability to emerge in the field. Critical root length for *Fagus sylvatica* was found to be 45 mm. The percentage of normally germinated seeds with roots longer than 45 mm in the CRL test is a predicted estimate of the field emergence percentage of a seed lot. Results of two tests on 5 and 10 seed lots showed generally good correlation between CRL predicted emergence and actually obtained field seedling emergence. Large variation in root length was found between and within seed lots, thus displaying large differences in seed vigour. The new test is an applied, easy and inexpensive vigour test developed for nurserymen and seed technicians in order to predict field emergence more accurately.

Additional key words: critical root length, CRL test, paper roll, predictability

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Introduction

Exact knowledge on the field emergence percentage of a seed lot is essential in determining sowing density to obtain a high quality seedling stand. However, traditional seed lot information, – like tetrazolium tests and laboratory germination under optimal conditions, – does not adequately describe field emergence capacity (Bonner 1974).

Assuming that seed dormancy of beech *Fagus sylvatica* seeds has been properly released, using for instance modern techniques of chilling seeds at controlled moisture content (Suszka and Zieta 1979; Muller and Bonnet-Masimbert 1989; Derkx 2001) the difference in germination in the laboratory and seedling emergence in the field may be caused by low seed vigour. Low vigour seeds may germinate under optimal conditions in the laboratory but may not survive in the field under more severe stress conditions. Hence, a vigour test is needed, that can predict field emergence accurately. In order for a standardised vigour test to be efficient, reliable and hence of interest to the grower, the field conditions during emergence should also be standardised as much as possible, so that variation in temperature, soil moisture and sowing methods is minimised between seed lots and years.

A number of different vigour testing methods, for example leachate conductivity (Bonner and Vozzo 1986; Bonner 1986; 1989), accelerated ageing (Blanche et al. 1990; Chaisurisri et al. 1993), exhaustion test (Bonner 1974), 'Triebkraft' test (Rohmeder 1951), seedling uniformity test (Huang 1989), fumarase enzyme activity (Shen and Oden 2000), seedling vigour classification (Chen et al. 1989) have been used to assess the seed vigour of tree species. Vigour tests can be used for assessment of a number of as-

pects of seed quality, for instance harvest or production quality, storage potential, stress tolerance during germination and for predicting field seedling emergence. The only previous research on vigour testing in beech seeds seems to be the work by Muller (Gosling 1995). She has done research on developing and testing an accelerated ageing vigour test on beech with the purpose of predicting storage potential of freshly harvested seeds. Publication of results is in preparation (C. Muller, INRA-Nancy, pers. com.). In the present paper vigour is only considered for accurate prediction of field emergence percentage.

Often the average or summed total of a vigour index is calculated for a complete sample of seeds or seedlings. This approach often gives problems in prediction accuracy as few extreme seed responses may affect the average significantly. The concept of studying seed lot vigour by measuring single seed responses as part of a population distribution is therefore gaining interest (Davidson and Moore 1994; Hampton 1995) since this should give more reliable predictions. Combining the population distribution of vigour responses with a critical threshold value for positive or negative evaluation of the potential of seedlings to emerge in the field (Barla-Szabo and Dolinka 1988; Davidson and Moore 1994; Hampton and Tekrony 1995) is a promising method that deserves more attention. The potential benefit for growers of such a method is that the critical threshold value can easily be adjusted for local conditions thus optimising an 'in-house' test.

The present study was therefore undertaken with the aim to develop a new, easy and reliable, practical method of testing field emergence potential of beech seeds. The concept of the new test is based on observations from preliminary experiments, which showed that 1) some seeds may germinate in the soil but they are not vigorous enough to penetrate the soil and emerge and that 2) root lengths of one-year old seedlings vary considerably both within and between seed lots. Furthermore an ability to produce rapid early root growth is extremely critical for the establishment and early resistance to moisture stress in the field. A highly vigorous hypocotyl or cotyledon growth will not be of any use if the root is not performing well. A number of results also document that radicle and initial root elongation is a very sensitive indicator of vigour in many species (Loeffler et al. 1985; Steiner et al. 1989; Bingham et al. 1994).

Our working hypothesis was consequently that possible differences in early root growth capacity in a laboratory test would be correlated to the field seedling emergence capacity of seed lots. In order to be able to measure and evaluate the root length of germinated beech seeds a method that allows an easy and fast inspection of roots was needed. The ISTA cold test for example in maize (Hampton and Tekrony 1995) is based on germination of seeds in soil in a vertically positioned paper roll, that when unfolded allows such an inspection of roots. Avoiding soil medium will make inspection even easier and improve reproducibility (Loeffler et al. 1985). A vertically positioned paper roll without medium has also been used in exhaustion tests (Bonner 1974) and in detailed studies of radicle elongation rates in maize seeds (Bingham et al. 1994). Beech seed normally produce a single primary root easy to evaluate and a paper roll test method therefore was adapted for testing beech seeds.

In the ISTA cool germination test, developed for cotton seed and also based on germination in paper rolls without medium, the combined length of primary roots and hypocotyls is measured after a specified test period and conditions (Hampton and Tekrony 1995). Instead of using the average length of roots-hypocotyls the distribution of individual lengths is combined with a critical root-hypocotyl length to provide a percentage good seedlings with longer roots than this. Smith and Varvil (1984) for example found a good correlation between the percentage of germinated cotton seedlings with a root-hypocotyl longer than 38 mm after 7 days at 18°C in a paper roll test and field emergence under cool soil conditions. A similar concept of a critical root length (excluding hypocotyl) was adapted for beech seed. Both test temperature, duration and critical root length need, however, to be adapted specifically to beech. The objective of this paper is to present 1) a modified vigour test method developed for beech seeds, 2) the basis for correlating to field emergence and 3) results of practical testing of the method. We have named the new test method for tree seeds the "Critical Root Length – CRL vigour test". To the authors knowledge this type of vigour test and criteria has not been investigated on tree seeds before.

Materials and methods

Laboratory set-up of the CRL test method

A standard filter paper for germination testing type AGF 614 (Frisenette Aps, Ebeltoft, Denmark) is cut into 28×56 cm in size and moistened briefly in sterile water and allowed to drain off for a few seconds. The paper is placed on a clean horizontal surface facing the longer side of the paper towards the person. Cold stratified, non-dormant and fully imbibed beech seeds are then positioned 3 cm from the top edge of the paper with their radicle end pointing downwards. 25 beechnuts (nutlets used synonymously with seeds in this paper) are placed on each sheet of paper. The paper is then rolled with the seed so tight that the seed is fixed between the paper when the paper roll is

placed vertically with the seed at the top end. A loose rubber band is placed around the 28 cm long roll to make sure that no seeds fall down during testing. Tightly bound rubber bands will create non-linear roots that are more difficult to measure. Two rolls each with 25 seeds placed in a 750 ml glass jar to keep the paper rolls upright constitutes one replicate of 50 seeds. Eight replicates of 50 seeds are used in testing vigour of individual seed lots. The jars are filled with 3 cm of sterile water, which creates a distance of 22 cm from the water surface to the seed. This provides an exact and constant humidity of the paper surrounding the seeds and growing roots which is important for high reproducibility.

Finally the paper rolls and jars are covered with a 0.025 mm thick plastic bag (LDPE size 250×500 mm) to provide an even humidity of the paper and enclosed air during the testing period. There is no need to add water during the 20 day test period.

This unit can then be placed in a germination cabinet or in a controlled temperature room. Replicate jars should be at randomised positions in the test facility. The seeds will germinate and the root will grow vertically straight downwards alongside the filter paper, making it easy to measure the length of roots after un-folding.

Test conditions adapted for beech seeds: temperature, duration, light

A number of preliminary investigations (not reported here) were made to determine the following conditions for beech. A constant temperature of 15 ± 0.5°C was adapted in order to simulate seed bed temperatures in spring, to allow a fast germination response and to avoid possible further release of primary dormancy or induction of secondary dormancy during testing. If conditions are very different in the field, either very cold or very warm, or if seeds have not been properly released from dormancy this test temperature may not apply with success. A 20-day duration of the test was adapted in order to provide as fast a test as possible but allow at least as many percentage seeds to germinate as would emerge in the field. A 12 hour daily light regime was adapted in order to make chlorophyll formation and greening of the hypocotyl possible, to allow a clear separation of root and hypocotyl tissue when recording only root length. Cool white fluorescent light from light tubes was used providing 50±5 mMol/m^2/s, but light intensity, exposure time and quality are not considered to be critical for satisfactory chlorophyll formation.

Evaluation of CRL test

After 20 days of germination the paper rolls are unfolded and individual seeds scored as normal germinated or not, and the root length of each seedling measured from the root tip to the root collar, as identified by the change in colour from white root tissue to green hypocotyl tissue. The root collar can normally be identified with a precision of ± 1 mm. Some late germinating seeds may only display root tissue and no hypocotyl tissue without the possibility of identifying the root collar. In these seeds the length from the root tip to the seed coat is measured. In seeds that develop two or more roots only the longest root is measured. During the development of the new method the root length of all germinated seeds was measured. However, in the applied final test only the number of normally germinated, sound seedlings that are able to produce a root longer than a critical root length of 45 mm is counted. This makes the recording and evaluation of the results fast and very easy in practice.

Evaluation of seeds contaminated by fungus can create some difficulties in some seed lots, since it is difficult to distinguish between primary infection and secondary infection spread from one seed to another during testing. The approach used in the present applied test, is to exclude germinated seeds with roots longer than 45 mm if they demonstrate a clear pathogenic attack by fungi or bacteria in either part of the seed, root, hypocotyl or cotyledons. In the case of heavily spreading fungi between seedlings during the CRL test this may give rise to erroneous lower estimates of field emergence, since the same spread of fungi may not occur in the field where seeds are not in close contact and conditions are not so optimal. On the other hand the test result in such cases clearly shows that this seed lot contains high-risk fungi that may create serious problems if not handled properly. All tests in the laboratory and in the field were done without the use of any fungicides or disinfectants.

Field emergence test

CRL tests and field sowing were investigated on 5 commercial seed lots of beech in 1999 and 10 seed lots in 2001. All seed lots in both years were from Danish officially approved forest provenances with the exception of one seed lot each year imported from Rumania. In the year 2001 five of the seed lots used were freshly harvested and other 5 seed lots were stored for two or three years. A sample of the same cold stratified seed lots, used for CRL testing in the laboratory, was sown in field seedbeds at the Department of Horticulture in early May following traditional methods applied in Danish nurseries. For every seed lot 4 replicates of 250 seeds were sown with 250 seeds in each 1 1 m plot. The seeds were covered with 1 cm layer of humid washed fine sand and protected from birds by green net (polyethylene Sanal 30, mesh size 0.5 0.5 cm, 75% light transmission, Hedeselskabet, Viborg, Denmark) on metal hoops 45 cm high and 100 cm wide, i.e. a seedbed tunnel. Plots for different seed lots were randomised

within 4 blocks. The seedbed was kept moist by irrigation during the first 2–3 weeks after sowing. The number of normally germinated sound seedlings was counted 50 days after sowing.

Concept of determination of the Critical Root Length

In order to determine the critical root length of a new species it is necessary to consider a combined data set of individual root length data from a CRL test and results of actual field emergence of the same seed lot. An example of the distribution of root lengths in three tested seed lots of *F. sylvatica* with 100 seeds each is shown in Fig. 1. Each dot shows the length of one root. The roots have been sorted with falling length to the right. Non-germinated seeds are marked as a 'zero'. Clear differences in germination percentage and length of roots is seen both between and within seed lots. Actual field emergence is normally always lower than laboratory germination. If we assume that seeds, producing only very short roots in the CRL test, will not be able to emerge in the field, whereas seeds that produce very long roots in the CRL test are very likely to emerge and establish in the field, we may define a critical minimum root length that correlates with the ability to emerge in the field. For example, if actual field emergence in population A (Fig. 1) was 81% this means that only the 81% seedlings with the longest roots are able to emerge in the field. The root length of the germinated seed No. 81 counted from the longest roots (the 'interception' between actual field emergence and CRL root length is 43 mm) describes the critical root length for this specific seed sample. A 49% field emergence in seed lot B equals a critical root length of 50 mm and 27% field emergence in seed lot C equals 41 mm root length. The mean critical root length determined over a number of seed lots and different years will provide the critical root length value, which gives the best prediction of field emergence. An initial estimate of the critical root length for *F. sylvatica* seeds was derived from tests of 8 replicates of each of 5 seed lots of different provenances compared to field emergence in spring 1999 at the Department of Horticulture. This critical root length value was then used to calculate CRL predicted emergence in the 5 seed lots from 1999 and to test 10 new seed lots of *F. sylvatica* in 2001.

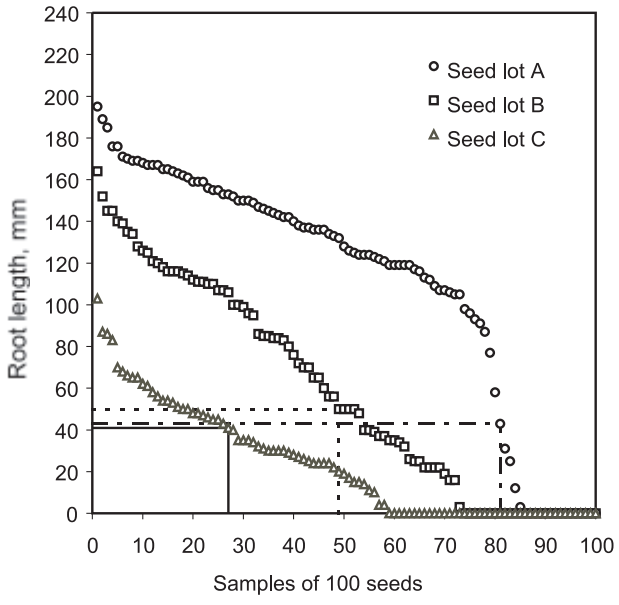

Fig. 1. Determination of the 'critical root length' based on the interception of the actual field emergence percentage and the recorded individual root length sorted with falling length to the right for three seed lots. Each dot represents the length of one primary root measured from the root collar to the root tip

Calculations and statistical analyses

Emergence percentage in the field was calculated as the mean and standard error of 4 replicates of 250 seeds. CRL test predicted emergence was calculated as the mean and standard error of 8 replicates of 50 seeds using the percentage of normal germinated seeds with roots longer than 45 mm as criterion. The numerical difference between predicted field emergence by the CRL test and actual field emergence was calculated for the 10 seed lots in 2001 as a measure of the error in prediction. The absolute difference between the average CRL test result and average actual field emergence of the 10 seed lots was also calculated in order to provide an overall estimate of whether and how much the CRL test overestimated or underestimated actual field emergence. Correlation coefficient and r^2 values between the CRL test predicted field emergence and actual emergence were calculated. Coefficient of variation was also calculated for each seed lot for the CRL test and the field emergence in the 2001 experiment.

Results

Variation in root length within and between paper rolls

Clear differences in germination and length of roots was found both within seed lots and between seed lots (Fig. 2, 3 and 4, here showing all seedlings including roots of seedlings killed by fungi). Root length after the 20 day CRL test varied from a few mm up to approximately 200 mm within seed lot No. 10 (Fig. 3) whereas maximum root length was less than 120 mm in seed lot No. 1 (Fig. 2). General root length obtained in individual replicates only varied little in seed lot No. 1 and in the longest roots of seed lot No. 10, suggesting that conditions for root elongation and growth did not vary significantly between replicates.

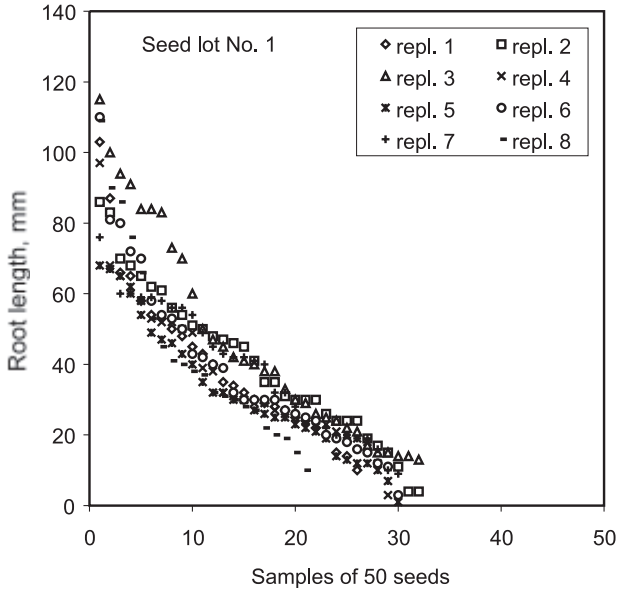

Fig. 2. Root length distribution of germinated seeds from each of 8 replicates from seed lot No. 1 based on 50 seeds per replicate. Each dot indicates the length of one seed in mm

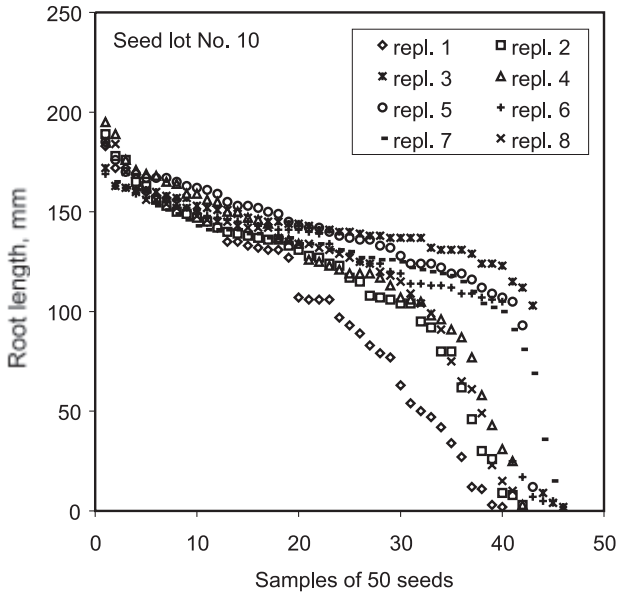

Fig. 3. Root length distribution of germinated seeds from each of 8 replicates from seed lot No. 10 based on 50 seeds per replicate. Each dot indicates the length of one seed in mm

The variation in root length between replicates in the 50% shortest roots in seed lot No. 10 is suggested to be caused by variation in the number of high and low vigour seeds in different replicates. The average root length of seedlings from the 10 different seed lots is shown in Fig. 4. Large differences are seen in the fraction of germinated seeds and the obtained root length of seedlings. The shapes of the distribution curves also vary from a reverse double sigmoid shape in high vigour seed lots to a hyperbolic shape in low vigour

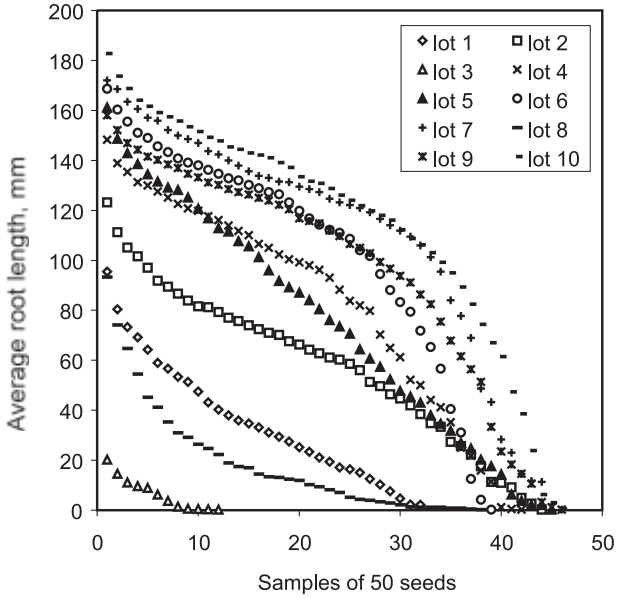

Fig. 4. Root length distribution in 8 different seed lots based on the average root length of 8 replicates of 50 seeds. Average length of seed No. 1 was calculated as the mean of the longest roots from each replicate, seed No. 2 as mean of the second longest roots, etc.

seed lots. Root length is clearly a very sensitive indicator of vigour in *F. sylvatica* seeds.

Critical root length

Based on comparisons of root length distribution and field emergence percentage in 5 seed lots of different provenances in spring 1999 (data not shown) a critical root length of 45 mm was estimated for *F. sylvatica* seeds. This estimate provided the best correlation between CRL predicted and actual field seedling emergence.

CRL testing of field seedling emergence in different seed lots of Fagus sylvatica

Results of the test in 1999 on 5 seed lots of *F. sylvatica* is shown in Fig. 5. As the critical root length estimate was derived from testing these seed lots, this means that the correlation in the 1999 test is partly biased and not independent. The standard error in the CRL test results for individual seed lots and the ability to predict the field emergence using a critical root length of 45 mm can, however, be evaluated. In general the CRL test results fits well with the field emergence in 4 out of 5 seed lots, suggesting that the use of 45 mm root length as criterion for positive evaluation of seedlings was acceptable in this experiment. The Pearson correlation coefficient for the 5 seed lots was 0.988 and the squared value 0.976 meaning that the CRL test could explain 97.6% of the variation in the field emergence. Seedlings in one seed lot did not produce roots long enough to give a positive CRL test result. The about 8% seedlings that had emerged in

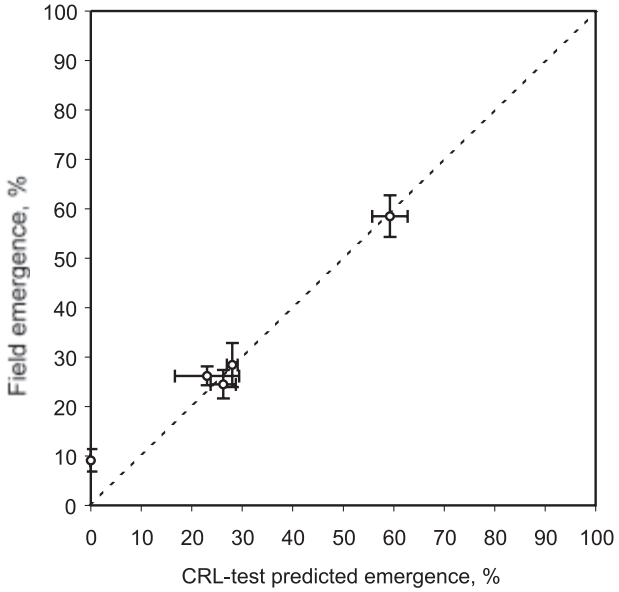

Fig. 5. Correlation between the predicted field seedling emergence by CRL test and the actually obtained field emergence in 5 seed lots in the spring of 1999. Data are mean and standard error of 8 replicates of 50 seeds in the CRL test and 4 replicates of 250 seeds in field emergence. The dotted line represents a 100 percent correlation between actual and predicted emergence

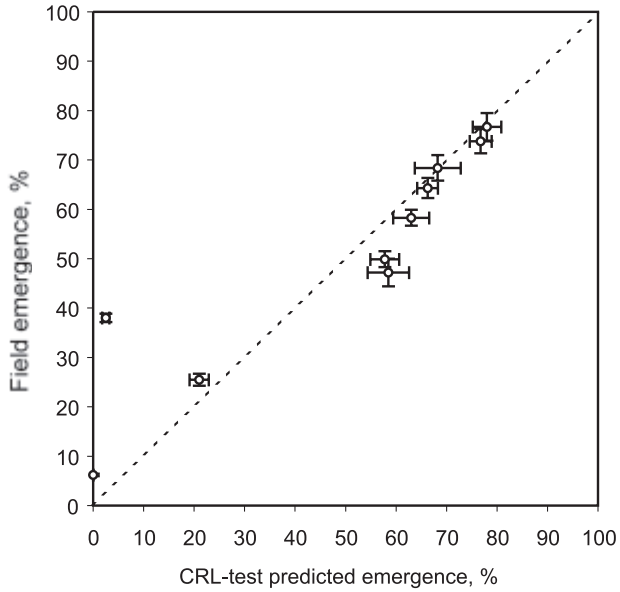

Fig. 6. Correlation between the predicted field seedling emergence by CRL test and the actually obtained field emergence of 10 seed lots of *Fagus sylvatica* in spring 2001. Data as in Fig. 5

the field after 50 days of this seed lot, were of low quality with short roots and did not all survive a full growing season. This suggests that quality of the emerged seedlings is an important parameter to consider in comparisons of test results and field emergence.

Results of the experiment on 10 new seed lots in spring 2001 are shown in Fig. 6. In this case the results are independent of the determination of the critical root length value. The ability to predict field seedling emergence is good in 9 out of 10 seed lots, with numerical differences between predicted and actual seedling emergence being less than 8% in 8 seed lots out of 10 (Table 1). Predictability in one seed lot (No. 8) was very poor due to a detrimental hyphal spread of fungi to almost all seeds during the paper roll testing, affecting both germination percentage and length of roots. Field emergence, however, was 38%. Excluding this seed lot the mean numerical deviance of the 9 other seed lots of the CRL test from the actual field emergence was 4.5%. The Pearson correlation coefficient was 0.98 and the squared value 0.965, meaning that the CRL test could explain 96.5% of the variation in the field seedling emergence. The absolute mean difference is 2.1%, which means that the CRL test on average overestimates field emergence by approximately 2%. This result confirms that a critical root length of 45 mm is very close to a best fit of the test also for the 2001 experiments.

Discussion

Root length

The large variation in root length between and within seed lots suggests that root length as measured in the CRL test is a very sensitive indicator of seed vigour and the performance of seeds during germination and establishment in the field. This confirms similar observations on other species that seedling radicle or root length is significantly correlated with vigour and field performance of seeds (Steiner et al. 1989). The root elongation rate and time of radicle protrusion has been shown to be the main governing factors for the root length obtained after a defined pe-

Table 1. Coefficients of variation of CRL test results and field emergence (FE), and numerical (*) or absolute mean (a) difference between CRL predicted field emergence and actual obtained field emergence percentage in 10 seed lots of beech in spring 2001. Seed lot No. 8 was excluded from mean differences

Seed lot	1	2	3	4	5	6	7	8	9	10	Avg.
CV of CRL	0.25	0.14	–	0.16	0.20	0.09	0.08	0.83	0.19	0.10	0.23
CV of FE	0.10	0.06	0.08	0.06	0.12	0.06	0.07	0.05	0.08	0.07	0.07
CRL test	–4,5	7,8	–6,2	4,7	11,3	1,9	2,9	–35,5	–0,15	1,3	4,5*
FE											2,1a

riod under defined conditions (Bingham et al. 1994). Comparisons based on mean root length or root weight has often resulted in intermediate correlation's to field germination (Bonner 1974; Huang 1989), since individual variation may affect the mean significantly. The difference in shape of the distribution curves for different seed lots presented here (Fig. 4) documents that such variation exists in beech. The relatively small variation in the obtained general root length in the 8 different replicates and the relatively low CV values of the CRL test on most seed lots (Table 1) indicate that standard conditions for root growth were easy to establish in different replicates and that reproducibility of the CRL test estimate is good between paper rolls within one laboratory. Further studies are undertaken to verify the reproducibility of the test results between different laboratories.

Correlation with field seedling emergence

The results document that the CRL test, using a 45 mm minimum root length as evaluation criterion for *F. sylvatica*, with the exception of one seed lot (year 2001), can explain more than 95% of the variation found in field emergence. This means that the CRL test correlated much better with field emergence percentage than a laboratory germination test on moist paper at constant 4°C recording only radicle protrusion or a tetrazolium test, that both overestimated germination capacity significantly (data not shown). Muller (INRA-Nancy, pers. com.) found that a seedling emergence test in soil in the laboratory was well correlated with field seedling emergence with a coefficient of correlation of more than 0.9, which suggest that laboratory seedling growth is a valuable measure of field performance in beech. Only the seedling shoot can, however, be easily evaluated in a seedling emergence test in medium, which means that quantitative information on the root performance normally will not be available. The critical root length of 45 mm is suggested to be valid in both years for a total of 15 seed lots, representing 10 different provenances either freshly harvested or stored for one or more years. Thus the new test method may be a very valuable tool in accurate prediction of the actual emergence percentage in the field, but may also be used to evaluate the general vigour of a seed lot. The seed lot No. 8, where the test failed to identify the field emergence percentage, was heavily attacked by a fungus spreading rapidly through paper rolls to all seeds during the test. Many seedlings reached a root length of more than 45 mm but were killed by the fungus in the last part of the 20 day's test and hence were not evaluated as sound seeds. A very high coefficient of variation of the CRL test (seed lot No. 8, Table 1) may indicate that fungus attack is present and hence interfere with the homogeneity of replicate results and general reliability of results. Using a fungicide during CRL testing may reduce the problems of fungal spread and thus reduce the variability of replicates and therefore improve the reliability of the test in such extreme seed lots. Also it is very important to release seed dormancy completely before CRL testing as semi-dormant seeds of beech may either not germinate at 15°C or show reduced root growth.

Field and nursery conditions

Since all field emergence trials was performed at one location by the same staff using the same methods and approximately similar sowing dates, the correlation is valid only for these conditions. Since field conditions, sowing methods and seed handling may vary between nurseries it is likely that field germination of a seed lot will be different in each nursery. Thus critical root length as determined in this study may be different for different nurseries due to local variation. Minor genetic differences in rate of root growth between seed lots from different provenances or geographic origin may also exist. It is, however, quite easy to adjust the critical root length up or down according to local experience, which means that the CRL test is versatile and easy to optimise in each company. This also implies that the CRL test may be most valuable as an 'in-house' test when the aim is to predict field emergence and optimal sowing density. Improved standardisation of nursery methods and field germination conditions will increase the value of such a test. Variable field conditions and methods in the company will always result in fluctuating predictability of any test for field emergence.

In conclusion the Critical Root Length vigour test is a promising method for predicting field emergence in tree seeds, but may also be of interest in other crops and for general estimates of seed vigour based on evaluation of root distribution curves. The CRL vigour test on *Fagus sylvatica* was developed as an applied, easy, versatile and non-expensive method for nurserymen and seed technicians to determine the potential field seedling emergence in tree seed lots prior to spring sowing. To the author's knowledge this is the first attempt to develop a seed vigour test in beech in order to predict field emergence percentage accurately.

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