

# Using the F<sub>3</sub> generation for predicting the breeding potential of soybean crosses

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## ABSTRACT

The prediction of the genetic potential of crosses for generating inbred lines scoring either above or below given standards can be obtained from the F<sub>3</sub> means and the variances among the F<sub>3</sub> families. The methodology was tested for two soybean (*Glycine max* L. Merrill) traits: a) plant height at maturity, of moderate to high heritability and b) grain yield, of low heritability. The predictions were helpful for screening among crosses regardless of the inheritance of the trait in the absence of genotype x environmental (g x e) effects. In the presence of g x e, the use of two standards and of data from two environments improved the yield predictions.

## INTRODUCTION

Estimation of the genetic potential of crosses is of great importance in breeding programs. When this prediction is made at the beginning of a program, less promising materials can be discarded before expensive yield trials are conducted.

Jinks and Pooni (1976) described a methodology to make predictions for the potential of a single cross to generate superior inbred lines, using information from the early cross generations. The distribution of the inbred lines derived without selection from a single cross is normal with mean  $m$  and genetic variance  $D$  (Mather and Jinks, 1982), when only additive and dominance effects control the characters being studied and when six or more genes are segregating in the parents. The knowledge of  $m$  and  $D$  is sufficient for predicting the proportion of the distribution of the random inbred lines scoring above or below any

standard the breeder may specify. These parameters can be estimated from the mean of the F<sub>3</sub> generation ( $m + 1/4 [h]$ ) and twice the variation among F<sub>3</sub> families [ $2 \times (1/2 D + 1/16 H)$ ] as estimates of  $m$  and  $D$ , respectively (Jinks and Pooni, 1980). The means and the variances of the F<sub>3</sub> families can easily be obtained in a breeding program using a simple experimental statistical design, as proposed for soybean by Toledo (1989).

This prediction methodology has been used in several situations with barley (*Hordeum vulgare* L.) (Tapsell and Thomas, 1983; Caligari *et al.*, 1985), with *Nicotiana rustica* (Pooni and Jinks, 1981) and with soybean (*Glycine max* L. Merrill) (Toledo, 1987, 1989; Triller, 1994). It was efficient even in the presence of directional dominance, epistasis, and linkage disequilibrium controlling the trait under analysis. The presence of the genotype x environment (g x e) interaction, however, has caused departures between the estimated and observed values (Toledo, 1986; Ooijen, 1989a,b). The objective of this study was to determine the applicability of the predictions in the presence of g x e effects.

## MATERIAL AND METHODS

Three soybean cultivars BR-13 (Bragg (4) x Santa Rosa), OCEPAR-8 (late mutant of cultivar Paraná), FT-2 (selection within cultivar IAS-5), and the experimental line BR85-29009 (União (6) x Lo 76-1763, which is a late flowering selection of the cultivar Industrial) were crossed in all (twelve) possible single cross combinations to obtain the  $F_2$ ,  $F_3$ ,  $F_8$ ,  $F_9$ , and  $F_{10}$  generations. After checking for the presence of reciprocal effects the data were pooled over reciprocals into six sets. The parents were chosen for their maturity, between 120 to 130 days from emergence to harvest, variable response to day-length variation (Hartwig and Kihl, 1979), which includes traditional (BR-13, FT-2 and BR85-29009) and late flowering under short-day environment (OCEPAR-8) genotypes (EMBRAPA, 1994), and high yield.

All experiments were conducted under field conditions at Londrina, PR, Brazil, at latitude 23°23'S. Sowings were done in November, within the recommended dates for soybean planting, and plants were kept under optimal growing conditions, including supplementary irrigation.

The mean and among individual variance of the parents and the  $F_3$  mean and the among and within family variance were obtained from field experiments conducted during three seasons (1992/93, 1993/94 and 1994/95). The means of the  $F_8$ ,  $F_9$ , and  $F_{10}$  families were available in 1992/93, 1993/94 and 1994/95, respectively. The number of parental individuals varied between 40 and 50 in the three experiments while 40  $F_3$  families, each including five plants, were available for every cross. The  $F_8$ ,  $F_9$ , and  $F_{10}$  generations included 100 families of four plants.

A completely random experimental design of single-plant hill plots was used. Hills were placed every 20 cm in rows spaced 1.5 m. Between the experimental rows, two border rows formed by a mixture of remnant  $F_2$  and  $F_3$  seeds were sown to equalize plant competition, obtain adequate plant density (about 250,000 plants/ha), and to facilitate data collection. The final row spacing in the experiment was 50 cm. Plant height at maturity (moderate to high heritability) and yield (low heritability) were selected for the estimates of the genetic potential of the crosses.

The methodology used for the predictions of the genetic potential of the crosses to generate inbred lines scoring either above or below standards was described by Jinks and Pooni (1976, 1980). The parental genotypes which, on average in the three years, had the greatest yield and the tallest and shortest plant height at maturity were used as standards for all crosses. The

average of the two highest yielding and the two tallest and two shortest parents were also used as standards. The proportion of  $F_8$ ,  $F_9$  or  $F_{10}$  families scoring either above or below the chosen standards was used to check the predictions.

The assessment of the reliability of the estimates of the breeding potential was determined by simple inspection and also by Pearson's correlation analyses (Snedecor and Cochran, 1967) between predicted and observed values. For each cross, the genetic potentials estimated in 1992/93, 1993/94 and 1994/95 were compared with the proportions observed in 1992/93 ( $F_8$ ), 1993/94 ( $F_9$ ) and 1994/95 ( $F_{10}$ ). When the comparisons between predictions and observations involved a single year (and consequently, a single experiment/year), the  $g \times e$  interaction effects were absent. Although these comparisons are of limited practical use to the breeder, they give valuable indications about the influence of the environment and its interactions with the genotypes on the predictions. Comparisons involving predictions and observations made in single different years had full impact from  $g \times e$  effects. Comparisons involving predictions calculated from two-year data, for example 1992/93 and 1993/94, and single year observations from 1994/95 were also used to lessen the  $g \times e$  interaction due to partial canceling of their effects, and to evaluate their reduced influence on the predictions and observations.

## RESULTS AND DISCUSSION

During the three-year period, the first and second highest-yielding parents were breeding line BR85-29009 (34.44 g/plant) and cultivar OCEPAR-8 (27.12 g/plant), respectively. The parental genotypes placed first and second for tall and short plant height at maturity were OCEPAR-8 (75.32 cm) and BR85-29009 (71.17 cm), and BR-13 (53.17 cm) and FT-2 (62.93 cm), respectively. They were chosen as standards for the breeding potential predictions and observations.

The presence of a complex genetic model controlling the traits analyzed was reported by Toledo *et al.* (1991). The presence of significant levels of  $g \times e$  interactions was detected by the joint (three years) analyses of variance of both traits (Table I).

Comparisons between predictions and observations realized in the absence of  $g \times e$  effects (i.e., conducted in a single experiment/year) indicated that the predictions were reliable for screening among crosses (Tables II and III). The magnitude of predicted and observed proportions of inbred lines scoring above or below the standards, however, was not always

**Table II** - Estimated (Est) and observed (Obs) genotypic potential of single soybean crosses to generate inbred lines yielding above the standards.

| Crosses    | Year                        | Standards for estimates |      |                     |      |
|------------|-----------------------------|-------------------------|------|---------------------|------|
|            |                             | ≥ BR85-29009            |      | ≥ (BR85+OCEPAR-8)/2 |      |
|            |                             | Est                     | Obs  | Est                 | Obs  |
| BR85-29009 | 1992/93/Obs F <sub>8</sub>  | 0.49                    | 0.46 | 0.67                | 0.59 |
| X          | 1993/94/Obs F <sub>9</sub>  | 0.35                    | 0.25 | 0.67                | 0.56 |
| FT-2       | 1994/95/Obs F <sub>10</sub> | 0.77                    | 0.63 | 0.75                | 0.63 |
| BR85-29009 | 1992/93/Obs F <sub>8</sub>  | 0.39                    | 0.36 | 0.47                | 0.45 |
| X          | 1993/94/Obs F <sub>9</sub>  | 0.22                    | 0.20 | 0.46                | 0.44 |
| BR-13      | 1994/95/Obs F <sub>10</sub> | 0.49                    | 0.51 | 0.47                | 0.52 |
| BR85-29009 | 1992/93/Obs F <sub>8</sub>  | 0.46                    | 0.38 | 0.58                | 0.51 |
| X          | 1993/94/Obs F <sub>9</sub>  | 0.40                    | 0.33 | 0.65                | 0.61 |
| OCEPAR-8   | 1994/95/Obs F <sub>10</sub> | 0.59                    | 0.53 | 0.57                | 0.49 |
| FT-2       | 1992/93/Obs F <sub>8</sub>  | 0.00                    | 0.11 | 0.02                | 0.14 |
| X          | 1993/94/Obs F <sub>9</sub>  | 0.06                    | 0.04 | 0.21                | 0.18 |
| BR-13      | 1994/95/Obs F <sub>10</sub> | 0.52                    | 0.44 | 0.49                | 0.41 |
| FT-2       | 1992/93/Obs F <sub>8</sub>  | 0.39                    | 0.32 | 0.49                | 0.42 |
| X          | 1993/94/Obs F <sub>9</sub>  | 0.07                    | 0.12 | 0.34                | 0.32 |
| OCEPAR-8   | 1994/95/Obs F <sub>10</sub> | 0.62                    | 0.55 | 0.60                | 0.52 |
| BR-13      | 1992/93/Obs F <sub>8</sub>  | 0.15                    | 0.19 | 0.26                | 0.26 |
| X          | 1993/94/Obs F <sub>9</sub>  | 0.10                    | 0.13 | 0.27                | 0.33 |
| OCEPAR-8   | 1994/95/Obs F <sub>10</sub> | 0.62                    | 0.48 | 0.59                | 0.46 |

**Table I** - Analysis of variance of the parental genotypes in the three growing seasons (years 1992/93, 1993/94, 1994/95).

| Source of variation      | df  | MS         |
|--------------------------|-----|------------|
| <b>(i) Yield</b>         |     |            |
| Year (Y)                 | 2   | 3256.81**  |
| Genotypes (G)            | 3   | 5026.12**  |
| Y × G                    | 6   | 1414.79**  |
| Error                    | 524 | 92.27      |
| <b>(ii) Plant height</b> |     |            |
| Year (Y)                 | 2   | 12235.24** |
| Genotypes (G)            | 3   | 17903.27** |
| Y × G                    | 6   | 337.92**   |
| Error                    | 519 | 66.07      |

\*\*Significant at 1% level.

**Table III** - Estimated (Est) and observed (Obs) genotypic potential of single soybean crosses to generate inbred lines taller or shorter than the standards.

| Crosses    | Year                        | Standards for estimates |      |         |      |                     |      |                  |      |
|------------|-----------------------------|-------------------------|------|---------|------|---------------------|------|------------------|------|
|            |                             | ≥ OCEPAR 8              |      | ≤ BR-13 |      | ≥ (BR85+OCEPAR 8)/2 |      | ≤ (FT-2+BR-13)/2 |      |
|            |                             | Est                     | Obs  | Est     | Obs  | Est                 | Obs  | Est              | Obs  |
| BR85-29009 | 1992/93/Obs F <sub>8</sub>  | 0.10                    | 0.21 | 0.06    | 0.01 | 0.16                | 0.30 | 0.10             | 0.05 |
| X          | 1993/94/Obs F <sub>9</sub>  | 0.21                    | 0.23 | 0.01    | 0.04 | 0.27                | 0.25 | 0.07             | 0.12 |
| FT-2       | 1994/95/Obs F <sub>10</sub> | 0.07                    | 0.28 | 0.02    | 0.05 | 0.15                | 0.37 | 0.14             | 0.21 |
| BR85-29009 | 1992/93/Obs F <sub>8</sub>  | 0.08                    | 0.16 | 0.30    | 0.08 | 0.12                | 0.20 | 0.36             | 0.10 |
| X          | 1993/94/Obs F <sub>9</sub>  | 0.17                    | 0.18 | 0.17    | 0.12 | 0.20                | 0.20 | 0.33             | 0.33 |
| BR-13      | 1994/95/Obs F <sub>10</sub> | 0.06                    | 0.20 | 0.29    | 0.20 | 0.10                | 0.30 | 0.52             | 0.34 |
| BR85-29009 | 1992/93/Obs F <sub>8</sub>  | 0.46                    | 0.53 | 0.03    | 0.02 | 0.54                | 0.63 | 0.04             | 0.02 |
| X          | 1993/94/Obs F <sub>9</sub>  | 0.51                    | 0.58 | 0.04    | 0.01 | 0.55                | 0.59 | 0.10             | 0.07 |
| OCEPAR 8   | 1994/95/Obs F <sub>10</sub> | 0.42                    | 0.48 | 0.03    | 0.03 | 0.52                | 0.59 | 0.11             | 0.09 |
| FT-2       | 1992/93/Obs F <sub>8</sub>  | 0.00                    | 0.04 | 0.41    | 0.26 | 0.00                | 0.04 | 0.54             | 0.34 |
| X          | 1993/94/Obs F <sub>9</sub>  | 0.04                    | 0.01 | 0.21    | 0.13 | 0.05                | 0.02 | 0.46             | 0.33 |
| BR-13      | 1994/95/Obs F <sub>10</sub> | 0.02                    | 0.12 | 0.24    | 0.16 | 0.04                | 0.21 | 0.55             | 0.31 |
| FT-2       | 1992/93/Obs F <sub>8</sub>  | 0.15                    | 0.27 | 0.02    | 0.03 | 0.24                | 0.38 | 0.03             | 0.04 |
| X          | 1993/94/Obs F <sub>9</sub>  | 0.27                    | 0.32 | 0.02    | 0.04 | 0.32                | 0.36 | 0.10             | 0.08 |
| OCEPAR 8   | 1994/95/Obs F <sub>10</sub> | 0.29                    | 0.39 | 0.03    | 0.03 | 0.41                | 0.46 | 0.11             | 0.09 |
| BR-13      | 1992/93/Obs F <sub>8</sub>  | 0.08                    | 0.18 | 0.13    | 0.09 | 0.12                | 0.22 | 0.19             | 0.11 |
| X          | 1993/94/Obs F <sub>9</sub>  | 0.09                    | 0.20 | 0.12    | 0.10 | 0.12                | 0.23 | 0.31             | 0.21 |
| OCEPAR 8   | 1994/95/Obs F <sub>10</sub> | 0.28                    | 0.21 | 0.10    | 0.07 | 0.36                | 0.35 | 0.24             | 0.11 |

**Table IV** - Correlations between estimated and observed proportions of the genetic potential of single crosses for generating inbred lines scoring above the standards for grain yield (g/plant).

| Year estimates      | Year observations | Standards            |                      |
|---------------------|-------------------|----------------------|----------------------|
|                     |                   | ≥ BR85-29009         | ≥ (BR85+OCEPAR-8)/2  |
| 1992/93             | 1992/93           | 0.98**               | 0.99**               |
|                     | 1993/94           | 0.83*                | 0.88*                |
|                     | 1994/95           | 0.85*                | 0.87*                |
| 1993/94             | 1992/93           | 0.79***              | 0.92**               |
|                     | 1993/94           | 0.96**               | 0.97**               |
|                     | 1994/95           | 0.62 <sup>n.s.</sup> | 0.75***              |
| 1994/95             | 1992/93           | 0.52 <sup>n.s.</sup> | 0.58 <sup>n.s.</sup> |
|                     | 1993/94           | 0.34 <sup>n.s.</sup> | 0.47 <sup>n.s.</sup> |
|                     | 1994/95           | 0.81*                | 0.76***              |
| (1992/93+1993/94)/2 | 1994/95           | 0.80***              | 0.77***              |
| (1992/93+1994/95)/2 | 1993/94           | 0.76***              | 0.84*                |
| (1993/94+1994/95)/2 | 1992/93           | 0.83*                | 0.91*                |

n.s., \*, \*\*, \*\*\* - Correlation not significant and significant at 5%, 1% and 10% level of probability, respectively.

similar, indicating that the methodology cannot precisely ascertain the exact proportions of desirable lines that will be derived from each cross. These findings were true for both yield and plant height. The correlation analyses between the predictions and observations confirm the previous results (Tables IV and V). This is in line with the findings of Jinks and Pooni (1976, 1980), Tapsell and Thomas (1983), Caligari *et al.* (1985), and Triller (1994), for different plant species.

The situation changed significantly when the comparisons between predictions and observations involved different years, which include  $g \times e$  (Tables II, III, IV and V). For yield, the interactions between the genotypes and the environments influenced the predicted and observed proportions of genotypes scoring above the standards and, consequently, lowered the correlation values between them. The influence of  $g \times e$  on the predictions was also recognized by Toledo (1986), working with *Nicotiana rustica*.

The interactions between genotype and environment can affect the data in three ways: acting on the standards, on the  $F_3$  generations used for the predictions and on the  $F_8$ ,  $F_9$  or  $F_{10}$  families used as observed results. Because  $g \times e$  interaction was present (Table I) and because predictions and observations are necessarily made in different environments, its effects had to be

reduced to obtain reliable results. The adverse effects were diminished when the average of two standards rather than only one standard was used. However, only small increases in the correlation values were observed. For final plant height predictions little improvement was obtained (Table V). On the other hand,  $g \times e$  effects were significantly reduced when predictions made in two years were averaged for comparison with the observations (Tables IV and V).

**Table V** - Correlations between estimated and observed genotypic proportions of inbred lines scoring above and below the standards for plant height (cm).

| Year estimates      | Year observations | Standards  |                      |                     |                      |
|---------------------|-------------------|------------|----------------------|---------------------|----------------------|
|                     |                   | ≥ OCEPAR 8 | ≤ BR-13              | ≥ (BR85+OCEPAR-8)/2 | ≤ (FT-2+BR-13)/2     |
| 1992/93             | 1992/93           | 0.99**     | 0.89*                | 0.98**              | 0.91*                |
|                     | 1993/94           | 0.97**     | 0.90*                | 0.98**              | 0.95**               |
|                     | 1994/95           | 0.89*      | 0.92**               | 0.97**              | 0.84*                |
| 1993/94             | 1992/93           | 0.97**     | 0.87*                | 0.98**              | 0.87*                |
|                     | 1993/94           | 0.97**     | 0.93**               | 0.96**              | 0.93**               |
|                     | 1994/95           | 0.95**     | 0.89*                | 0.95**              | 0.65 <sup>n.s.</sup> |
| 1994/95             | 1992/93           | 0.84*      | 0.70 <sup>n.s.</sup> | 0.85*               | 0.79***              |
|                     | 1993/94           | 0.85*      | 0.89*                | 0.88*               | 0.98**               |
|                     | 1994/95           | 0.82*      | 0.99**               | 0.90*               | 0.90*                |
| (1992/93+1993/94)/2 | 1994/95           | 0.93**     | 0.92**               | 0.96**              | 0.78***              |
| (1992/93+1994/95)/2 | 1993/94           | 0.95**     | 0.92**               | 0.97**              | 0.97**               |
| (1993/94+1994/95)/2 | 1992/93           | 0.97**     | 0.79***              | 0.98**              | 0.84*                |

n.s., \*, \*\* and \*\*\* - Correlation not significant and significant at 5%, 1% and 10% level of probability, respectively.

These reductions in the  $g \times e$  effects, obtained by averaging the two standards and when data from two years were considered, suggest that the predictions can be very useful. This is particularly true if one considers that the agreement between predictions and observations will, in practice, be higher because the observed results used for comparison were single-year data, which were affected by  $g \times e$  interaction.

Alliprandini *et al.* (1994) reported that years and locations produce similar effects in the performance of soybean genotypes grown in Paraná State, Brazil. This probably also happens elsewhere. Under these conditions, breeders can use information from two or more locations to obtain reductions in the  $g \times e$  biases while conducting their breeding programs, and abbreviate the length of time necessary to make decisions. Considering the reliability of the prediction procedures and the practicality of conducting the  $F_3$  progeny testing within the routine of a breeding program (Toledo, 1989), breeders should examine the potential of their crosses for developing superior cultivars.

## CONCLUSIONS

The methodology used in this work was efficient to predict the genetic potential of single crosses for high and low heritability traits, in the absence of  $g \times e$  effects.

In the presence of  $g \times e$  effects, the predictions of the breeding potential have a higher chance to increase the success of breeding programs if measures to reduce the  $g \times e$  biases are taken, specially for a low heritability trait such as yield. Data from two years (or environments) and two or more standards seem to adequately correct the  $g \times e$  biases.

## ACKNOWLEDGMENTS

The authors are grateful to Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq for the grants received during the period this work was carried out.

## RESUMO

A previsão do potencial genético de cruzamentos para gerar linhas puras com desempenho superior ou inferior a referências escolhidas pode ser realizada usando a média e a variância entre famílias da geração  $F_3$ . A metodologia foi testada para dois caracteres em soja: a) altura de planta na maturação, de herdabilidade moderada a alta, e b)

produtividade, de baixa herdabilidade. As previsões foram úteis para selecionar entre cruzamentos, independente da complexidade do controle genético do caráter e da sua herdabilidade, na ausência de interação entre genótipo e ambiente. Na presença de interação, o uso de mais de uma referência e, principalmente, de dados de dois ambientes foi útil para aumentar a precisão das estimativas para o caráter produtividade.

## REFERENCES

- Alliprandini, L.F., Toledo, J.F.F., Fonseca Jr., N.F., Almeida, L.A. and Kiihl, R.A.S. (1994). Efeitos da interação genótipo  $\times$  ambiente sobre a produtividade da soja no Estado do Paraná. *Pesq. Agropec. Bras.* 29: 1433-1444.
- Caligari, P.D.S., Powell, W. and Jinks, J.L. (1985). The use of double haploids in barley breeding. 2. An assessment of univariate cross prediction methods. *Heredity* 54: 353-358.
- EMBRAPA (1994). *Recomendações Técnicas para a Cultura da Soja no Paraná, 1994/95*. Boletim Técnico No. 79, Londrina, PR, pp. 61-88.
- Hartwig, E.E. and Kiihl, R.A.S. (1979). Identification and utilization of a delayed flowering character in soybean for short day condition. *Field Crops Res.* 2: 145-151.
- Jinks, J.L. and Pooni, H.S. (1976). Predicting the properties of recombinant inbred lines derived by single seed descent. *Heredity* 36: 253-266.
- Jinks, J.L. and Pooni, H.S. (1980). Comparing prediction of mean performance and environmental sensitivity of recombinant inbred lines based upon  $F_3$  and triple test cross families. *Heredity* 45: 305-312.
- Mather, K. and Jinks, J.L. (1982). *Biometrical Genetics*. 3rd edn. Chapman and Hall, London, pp. 396.
- Ooijen, J.W. Van (1989a). The predictive value of quantitative genetic parameters in autogamous crops: bias caused by intergenotypic competition. 1.  $F_{\infty}$ -mean. *Euphytica* 44: 81-94.
- Ooijen, J.W. Van (1989b). The predictive value of quantitative genetic parameters in autogamous crops: bias caused by intergenotypic competition. 2.  $F_{\infty}$ -variance. *Euphytica* 44: 95-108.
- Pooni, H.S. and Jinks, J.L. (1981). Sources of predictions and their reliability in predicting the properties of recombinant inbred lines which can be obtained from a cross by single seed descent. *Proceedings of the Fourth International Barley Genetics Symposium*. Edinburgh, Scotland. pp. 73-78.
- Snedecor, G.W. and Cochran, W.G. (1967). *Statistical Methods*. The Iowa State University Press, Ames, Iowa, pp. 593.
- Tapsell, C.R. and Thomas, W.T.B. (1983). Cross prediction studies on spring barley. 2. Estimation of genetical and environmental control of yield and its component characters. *Theor. Appl. Genet.* 64: 353-358.
- Toledo, J.F.F. (1986). Biometrical studies of inbred lines and their hybrids. Doctoral thesis, University of Birmingham, Birmingham.

- Toledo, J.F.F.** (1987). Predicting the inbreeding and the outcrossing potential of soybean [*Glycine max* (L.) Merrill] varieties. *Braz. J. Genet.* 10: 543-558.
- Toledo, J.F.F.** (1989). Quantitative genetics in soybean breeding. In: *World Soybean Research Conference. IV* (Pascale, A.J., ed.). Soybean Association of Argentina, Buenos Aires, pp. 909-914.
- Toledo, J.F.F., Rossini, M.C., Souza, R.F. and Leão, F.F.** (1991). Genetical analysis of a soybean biparental cross and comparative model fitting to means and variances of two sets of generations. *Braz. J. Genet.* 14: 1041-1064.
- Triller, C.** (1994). Previsão do potencial genético de cruzamentos em soja pela geração F<sub>3</sub>. Master's thesis. Universidade Estadual de Londrina, Londrina, PR, Brazil.

(Received September 12, 1995)