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CONSUMPTION GROWTH AS A MEASURE OF COMPARISONS OF RESULTS FROM NO-CHOICE TEST AND TEST WITH MULTIPLE CHOICE

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Abstract: Studies on food preference of herbivores include no-choice test and test with choice or multiple choice. Conclusions from statistic analyses of these tests are compared descriptively. The definition of compatibility index and consumption growth index has enabled us to use nonparametric test for verification of hypotheses about homogeneity of the consumption growths of selected plant species under no-choice and multiple choice conditions. The studies were conducted on food preference of the slug *Deroceras reticulatum*. It has been found that *Chamaenerion angustifolium*, *Geranium pusillum* and *Potentilla anserina* can be used to reduce this slug feeding on cultivated plants. It has been also found that seedlings of *Polygonum aviculare* can be used as alternative food for slugs.

Key words: compatibility index, index of consumption growth, nonparametric test, no-choice test, test with choice, test with multiple choice, *Deroceras reticulatum*

INTRODUCTION

Studies on herbivore food preferences are conducted as no-choice or/and choice tests. Tests with choice are mostly conducted on two species of tested plants. When studies are performed on at least three plant species, they are called tests with multiple choice. Measurements of rate of slug preferences for food in no-choice tests and in tests with choice as well as in tests with multiple choice were reported by Kozłowska and Kozłowski (2002). They are: the acceptability index enabling separation of plant species, which are more than averagely accepted or unaccepted by slugs, the palatability index making it possible to evaluate food preference for different plant species in relation to the control plant and the consumption index, which determines mean consumption of plants.

Indices proposed in the literature permit to evaluate food preferences under certain conditions (Cook et al. 1996; Kozłowski and Kozłowska 2000; Watkins et al. 1996). Investigations on food preferences of slugs are carried out in a laboratory using different kinds of containers. Depending on the number of simultaneously tested plant species, the applied containers are of suitable size. The size of container limits the number of simultaneously tested plant species and determines the number of slugs placed in it. As the number of studied plant species increases, the possibility of accidents also increases (Cook et al. 1997). This problem may occur when there is a group of plant species with a similar attractiveness among the plants under consideration. The sequence of seedling consumption of these species may be accidental. This indicates that plants of one species (for example A species) may be damaged first, then plants of the next species (for example B species). In this situation, despite a similar attractiveness of these species, the A species could be classified to belong to more acceptable species than the B species. To counteract the problem of accidents in the tests with multiple choice, several to a dozen or so slugs are placed into each container. It has been found that the increase of replication number and several to a dozen or so herbivores placed into each container effectively prevent the occurrence of the problem of accidents. The ratio of the number of studied plant species to the number of slug individuals is also important.

Selection of seedling number of each studied plant species in one replication is undoubtedly important as well. It is especially important in no-choice tests. In these studies, a single slug is placed as a rule in one replication and a visual estimation of seedling damages is used. The scale of the visual estimation is determined by selection of the seedling number. The number of seedlings should not be too large, however, it should satisfy food requirements of a herbivore. The scale of visual estimation is directly proportional to the number of seedlings of one plant species in a single replication. This indicates that the planned number of seedlings of one plant species in one replication can be higher, when the degree of the selected scale of plant damage visual estimation is higher.

The specificity of no-choice tests and tests with multiple choice determines formulas of acceptability, palatability and consumption indices for estimation of food preferences (Kozłowska and Kozłowski 2002). The values of these indices are different for the studied plant species in the both tests and the interpretation of them is different. Then a question arises: in what respect and in which way can the results of no-choice test be compared to those of the test with multiple choice?

The aim of this paper was to define the compatibility index of no-choice test and test with multiple choice conducted on the same collection of plant species with the same number of slug individuals per unit of the surface measure. As a common measure of food preference estimation for no-choice test and test with multiple choice, the consumption growth index has been proposed. The mean consumption growth has been obtained from the geometric mean formula. Nonparametric tests have been proposed to compare the means consumption growth (Friedman 1937; Kruskal and Wallis 1952; Mann and Whitney 1947). The proposed analysis has been carried out for the studies of *Deroceras reticulatum* food preferences.

Compatibility index for no-choice test and tests with multiple choice

Parameters of no-choice test and test with multiple choice include: s – the number of slug individuals placed on the studied surface (in a container), p – the number of seedlings on the studied surface, d – the number of feeding days and t – the number of tested plant species. It is important to maintain proportions between the number of feeding slugs and the studied surface (of a container) as well as with the total number of seedlings on the studied surface. On account of a different surface under study (soil surface in the container), the tests carried out on the same collection of plant species with a similar number of slug individuals per surface unite differ by the number of slugs in the container and by the number of seedlings in it.

Let us introduce the compatibility index of the form:

$$I_C = \frac{p_1 d_1 s_2}{p_2 d_2 s_1},$$

where p_1, p_2 denote the number of seedlings in the container in no-choice test and in test with multiple choice, respectively, d_1, d_2 denote the number of days from the beginning of test to the day of observation in no-choice test and in test with multiple choice, respectively, where s_1 and s_2 show the number of slug individuals in a single container in no-choice test and in test with multiple choice, respectively. Let us notice that p_1 is the number of seedlings of a single plant species in the container in no-choice test, whereas p_2 is the total number of seedlings of all plant species in the container, studied in the test with multiple choice.

If the index $I_C=1$, then no-choice test and test with multiple choice are comparable with regard to the mean consumption growth caused by slugs to seedlings of the studied plant species**.** The consumption growth index has the form:

$$CG.I_{(ijk)} = \begin{cases} 1+\dfrac{T_{ijk}}{100} & \text{for } j=1 \text{ or if } T_{i(j-1)k}=0 \\ \dfrac{T_{ijk}}{T_{i(j-1)k}} & \text{for } T_{i(j-1)k} \neq 0 \end{cases},$$

where T_{ijk} denotes the consumed mass (surface) of seedlings of i tested plant species (presented in percent) after j days of feeding in k replication, i=1,2,...,t, j=1,2,...,d, k=1,2,...,r (t – the number of the studied plant species, d – the number of days of the conducted research, r – the number of replications of a plant species). The mean consumption growth for two, three, four and the next day number of slug feeding (shortly speaking, for j feeding days) is estimated from the formula for geometric mean, that is

$$\overline{CG.I}_{(ijk)} = \sqrt[j]{\prod_{o=1}^{j} CG.I_{(iok)}}.$$

Because of the lack of information on distribution of the analysed variable (of the mean consumption growth) for verification of the hypotheses equating to at least two populations, it is proposed to use nonparametric tests: Wilcoxon's test, Kruskal's-Wallis's test or Friedman's test.

MATERIAL AND METHODS

Studies concerning preferences of the slug *D. reticulatum* for 20 plant species were carried out as no-choice test and tests with multiple choice under controlled conditions, at the day temperature of 19°C and night temp. of 16°C, RH humidity of 93% and 15 h day length.

No-choice tests were conducted in translucent plastic containers (dimensions 22 × 18 × 13 cm). The containers had small ventilation holes and were filled with a 5-cm soil layer. Seeds of one of the 20 plant species under consideration were sown in the container on the soil surface of 396 cm^2. At the stage of 2–3 leaves (seedling height 5–8 cm), 5 seedlings were left in the container and a single slug was placed on each of them. The slugs were starved for 48 hours. Once a day during 15 consecutive days, the percent of plant surface consumed by the slugs was determined using visual estimation acc. to a 5-degree scale (0% – lack of damages, 25%, 50%, 75% and 100%). Each time 5 seedlings of 20 plant species were tested in 10 replications.

Tests with multiple choice were conducted in translucent plastic closed containers (dimensions 80 × 50 × 20 cm). The containers were filled with soil (about 7- cm layer) to 1/3 of their height and divided into 40 plots. The containers had two ventilation holes protected with mill gause. Seeds of 19 herb species and oilseed rape were sown into these containers according to a scheme of complete block design. Because of differences in the rate of individual plant species development (determined on the basis of earlier observations of germination), their sowing dates were different to obtain possibly the most equalized plant material. When the plants (5–8 cm high) reached the stage of 2–3 leaves, 5 seedlings of one plant species were left on each plot. Then 10 slugs starved for 48 hours were placed in center of each container on surface of 4000 cm^2 soil. Over 30 consecutive days, the percentage of plant area eaten by the slugs was determined every day according to a 5-degree scale. Observations of damages were conducted on 5 seedlings in 6 replications for each of the 20 tested plant species.

RESULTS AND DISCUSSION

In no-choice tests, from the second to the seventh day of *D. reticulatum* slug feeding the highest consumption growth was found in the case of *Chrysanthemum parthenium* L. seedlings (Tab. 1). Also a high average consumption growth was found for the seedlings of *Matricaria chamomilla* L., *Satureja hortensis* L. and *Coriandrum sativum* L. The slugs did not feed or only attempted to feed on the seedlings of *Chamaenerion angustifolium* (L.) Scop., *Potentilla anserina* L., *Borago officinalis* L., *Impatiens balsamina* L. and *Geranium pusillum* L. Based on Kruskal's-Wallis's test it was found that highly significant differences occurred between the mean consumption growth of seedlings of the 20 plant species tested under conditions of no-choice test (Tab. 1).

In the tests with choice after the second analysed date of the studies (after day 8^{th} of *D. reticulatum* slug feeding) the highest average consumption growth was found for the seedlings of *C. sativum* L., *Brassica napus* L. var. *oleifera* L. and *S. hortensis* (Tab. 2). Over the entire period of studies, the slug did not feed on the seedlings of

Table 1. Mean consumption growth in no-choice test on successive dates of the studies, rank sums for consumption growth 7 days after and the result of Kruskal's-Wallis's test

Plant species	Number of days						Rank sums
	2	3	4	5	6	7	7 days
Achillea millefolium L.	1.00	1.01	1.06	1.08	1.09	1.11	885.5
Artemisia dracunculus L.	1.35	1.26	1.22	1.20	1.19	1.19	1287.0
Borago officinalis L.	1.00	1.00	1.00	1.00	1.00	1.00	334.0
Brassica napus L. var. *oleifera* L.	1.11	1.24	1.27	1.24	1.23	1.23	1430.5
Calamintha vulgaris (L.) Druce	1.01	1.10	1.09	1.08	1.07	1.06	739.5
Calendula officinalis L.	1.08	1.09	1.08	1.09	1.08	1.08	885.5
Chamaenerion angustifolium (L.) Scop.	1.00	1.00	1.00	1.00	1.00	1.00	373.5
Chrysanthemum parthenium (L.) Bernh.	1.57	1.56	1.43	1.35	1.34	1.31	1621.5
Coriandrum sativum L.	1.41	1.44	1.40	1.39	1.33	1.29	1542.5
Geranium pusillum L.	1.00	1.01	1.00	1.00	1.00	1.01	428.5
Helichrysum arenarium (L.) Moench.	1.22	1.22	1.25	1.23	1.22	1.21	1334.0
Impatiens balsamina L.	1.00	1.00	1.00	1.00	1.00	1.00	334.0
Malva silvestris L.	1.05	1.02	1.01	1.01	1.01	1.02	511.5
Matricaria chamomilla L.	1.46	1.36	1.28	1.23	1.19	1.18	1222.5
Mentha piperita L.	1.17	1.30	1.26	1.26	1.22	1.21	1313.5
Ocimum basilicum L.	1.01	1.11	1.17	1.17	1.15	1.14	1043.0
Polygonum aviculare L.	1.01	1.06	1.17	1.20	1.30	1.30	1463.0
Potentilla anserina L.	1.00	1.00	1.00	1.03	1.03	1.04	555.5
Salvia officinalis L.	1.39	1.46	1.39	1.33	1.32	1.31	1586.0
Satureja hortensis L.	1.43	1.36	1.26	1.21	1.18	1.16	1209.0
Geometric means and the value of statistic	1.15	1.17	1.16	1.15	1.14	1.14	117.819**

**highly significant differences

C. angustifolium and *P. anserina*. From the comparison of average consumption growths of seedlings of the provided 20 plant species after 28 days of the slug feeding, highly significant differences have been found between the compared populations (Friedman's test), (Tab. 2).

Comparing the results concerning average consumption growths of seedlings of particular plant species in the no-choice test with the average consumption growth in the test with multiple choice, it has been observed that differences occurred in the consumption rate. These tests can be compared because they were conducted on the same collection of 20 plants species and because in the both tests a single slug was placed on about a 4000 cm^2 soil surface in the container. In order to compare these tests, it should be found for which values (of the parameters p_1, d_1, s_1 of no-choice test and of the parameters p_2, d_2 and s_2 of the test with multiple choice) does the compatibility index assume the value 1. For the reason that in no-choice test a single slug ($s_1=1$) feeds on five seedlings ($p_1=5$) of one plant species growing on the area of 396 cm^2 soil in the container, the number of feeding days d_1 should be fixed. In the test with multiple choice, ten slugs ($s_2=10$) were placed in the container with the soil surface of 4000 cm^2, and five seedlings per plot grew on 40 plots in the container, i.e. $p_2=5*40=200$. Therefore, the number of the slug feeding days d_2 should be specified. Because no-choice test was conducted during 15 days and

Table 2. Mean consumption growth in test with multiple choice on successive dates of the studies, rank sums for consumption growth 7 days after and the result of Friedman's test

Plant species	Number of days						Rank sums
	8	12	16	20	24	28	28 days
Achillea millefolium L.	1.18	1.18	1.14	1.12	1.11	1.09	79.0
Artemisia dracunculus L.	1.00	1.00	1.00	1.08	1.08	1.08	72.5
Borago officinalis L.	1.13	1.13	1.11	1.13	1.11	1.10	90.0
Brassica napus L. var. *oleifera* L.	1.29	1.19	1.14	1.11	1.09	1.08	61.5
Calamintha vulgaris (L.) Druce	1.00	1.02	1.01	1.01	1.01	1.02	18.0
Calendula officinalis L.	1.16	1.18	1.14	1.12	1.11	1.10	89.0
Chamaenerion angustifolium (L.) Scop.	1.00	1.00	1.00	1.00	1.00	1.00	13.0
Chrysanthemum parthenium (L.) Bernh.	1.01	1.03	1.05	1.05	1.06	1.08	66.5
Coriandrum sativum L.	1.31	1.23	1.18	1.14	1.12	1.10	86.5
Geranium pusillum L.	1.00	1.00	1.02	1.03	1.03	1.03	32.5
Helichrysum arenarium (L.) Moench.	1.20	1.16	1.13	1.11	1.10	1.08	69.5
Impatiens balsamina L.	1.00	1.00	1.01	1.05	1.08	1.08	74.0
Malva silvestris L.	1.06	1.08	1.06	1.06	1.07	1.08	60.5
Matricaria chamomilla L.	1.19	1.17	1.14	1.12	1.10	1.09	80.5
Mentha piperita L.	1.00	1.00	1.01	1.03	1.05	1.05	42.5
Ocimum basilicum L.	1.21	1.17	1.15	1.12	1.11	1.10	80.0
Polygonum aviculare L.	1.00	1.01	1.13	1.15	1.12	1.11	92.5
Potentilla anserina L.	1.00	1.00	1.00	1.00	1.01	1.02	19.0
Salvia officinalis L.	1.07	1.07	1.05	1.05	1.06	1.07	56.5
Satureja hortensis L.	1.23	1.19	1.16	1.13	1.11	1.09	76.5
Geometric means and the value of statistic	1.10	1.09	1.08	1.08	1.08	1.07	57.090**

**highly significant differences

the test with multiple choice was conducted during 30 days, the compatibility index $I_C = 1$ for $d_1=7$ and for $d_2=28$.

Verification of hypotheses H_{0i} equating the average consumption growth of seedlings of i plant species under conditions of no-choice test to an average consumption growth of seedlings of the same plant species under conditions of test with multiple choice was conducted by Wilcoxon's test, i=1,2,...,20 (Tab. 3). Highly significant differences have been found for seven plant species, significant differences – for five species and no significant differences – for the remaining eight species.

Seedlings of such plant species, as *I. balsamina, B. officinalis* and *Malva silvestris* L., which were scarcely eaten in no-choice test and previously not eaten in the test with multiple choice, but after 28 days the mean consumption growth amounted to 1.08, 1.10 and 1.08, respectively. Such behaviour indicates that after a long feeding and multiple tasting of different plant species, slugs can feed on unacceptable plants. This is confirmed by the hypothesis that some herbivores (including slugs) are able to tolerate unacceptable plants and can feed on them causing damages (Whelan 1982). On the other hand, unaccepted food tasting permits slugs to recognize its smell and taste, and a result – to avoid it (Whelan 1982; Cook et al. 1997).

In the case of seedlings of *S. hortensis, C. sativum*, *Salvia officinalis* L., *C. parthenium* and oilseed rape, the average consumption growth in no-choice test was signifi-

Table 3. Rank sums and results of Wilcoxon's test for comparisons of consumption growth index under different conditions of food choice (no-choice and with multiple choice) on the 7th analysed date of the studies

Plant species	CG. index		Rank sums		Values of statistic
	no-choice	with choice	no-choice	with choice	
Achillea millefolium L.	1.11	1.09	84.0	52.0	0.054
Artemisia dracunculus L.	1.19	1.08	106.0	30.0	2.224*
Borago officinalis L.	1.00	1.10	55.0	81.0	3.200**
Brassica napus L. var. *oleifera* L.	1.23	1.08	109.0	27.0	2.549**
Calamintha vulgaris (L.) Druce	1.06	1.02	88.0	48.0	0.271
Calendula officinalis L.	1.08	1.10	72.0	64.0	1.356
Chamaenerion angustifolium (L.) Scop.	1.00	1.00	94.0	42.0	0.922
Chrysanthemum parthenium (L.) Bernh.	1.31	1.08	114.5	21.5	3.145**
Coriandrum sativum L.	1.29	1.10	111.0	25.0	2.766**
Geranium pusillum L.	1.01	1.03	76.0	60.0	0.922
Helichrysum arenarium (L.) Moench.	1.21	1.08	106.0	30.0	2.224*
Impatiens balsamina L.	1.00	1.08	61.0	75.0	2.549**
Malva silvestris L.	1.02	1.08	62.5	73.5	2.386*
Matricaria chamomilla L.	1.18	1.09	104.0	32.0	2.007*
Mentha piperita L.	1.21	1.05	107.0	29.0	2.332*
Ocimum basilicum L.	1.14	1.10	89.0	47.0	0.380
Polygonum aviculare L.	1.30	1.11	103.0	33.0	1.898
Potentilla anserina L.	1.04	1.02	89.0	47.0	0.380
Salvia officinalis L.	1.31	1.07	114.0	22.0	3.091**
Satureja hortensis L.	1.16	1.09	111.0	25.0	2.766**

*significant differences
**highly significant differences

cantly higher than in the test with multiple choice. This indicates that the neighbourhood of fifteen other plant species in the test with multiple choice limited the consumption of the mentioned plant species. The little smaller differences have been found for the seedlings of such species as *Artemisia dracunculus* L., *Mentha piperita* L., *Helichrysum arenarium* (L.) Moench. and *M. chamomilla* L.

Among the remaining species, for which the average consumption growth was similar in the both tests, *C. angustifolium*, *G. pusillum*, *P. anserina* and *Polygonum aviculare* L. deserve special attention. Seedlings of the first three plant species were not consumed in no-choice test as well as in the test with multiple choice. However, seedlings of *P. aviculare* in the both tests were readily consumed and the mean consumption growth was similar (Tab. 3). These species can be used in agricultural practice as an alternative method of slug control.

CONCLUSIONS

1. The mean consumption growth is a measure of food preference estimation with a similar formula for no-choice test and for test with multiple choice.
2. The application of compatibility index permits to compare results of consumption growth in no-choice test with the mean consumption growth in the test with multiple choice.

3. The application of Wilcoxon's test has made it possible to designate three groups of 20 studied plants species: 7 species with highly significant differences, 5 species with significant differences and 8 species with a similar mean consumption growth under conditions of no-choice test and test with multiple choice.
4. Seedlings of such species as *C. angustifolium*, *G. pusillum* and *P. anserina* can be used to reduce slug feeding on cultivated plants.
5. Seedlings of *P. aviculare* can be used as alternative food of slugs.
6. The proposed statistical analysis has made it possible to formulate total proposals from the both, no-choice test and test with multiple choice.

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POLISH SUMMARY

PRZYROST SPOŻYCIA MIARĄ PORÓWNANIA WYNIKÓW Z TESTÓW BEZ WYBORU I Z WIELOKROTNYM WYBOREM

Badania nad preferencją pokarmową roślinożerców obejmują testy bez wyboru i testy z wyborem lub z wielokrotnym wyborem. Wnioski z analiz statystycznych tych doświadczeń są porównywane opisowo. Zdefiniowanie wskaźnika kompatybilności i wskaźnika przyrostu spożycia pokarmu roślinnego umożliwia zastosowanie testu nieparametrycznego Wilcoxona do weryfikacji hipotez o jednorodności średnich przyrostów spożycia roślin wybranego gatunku w warunkach bez wyboru ze spożyciem go w warunkach wielokrotnego wyboru. Przeprowadzono badania nad preferencją pokarmową ślimaka pomrowika plamistego (*Deroceras reticulatum*). Wykazano, że wierzbówka kiprzyca (*Chamaenerion angustifolium*), bodziszek drobny (*Geranium pusillum*) i pięciornik gęsi (*Potentilla anserina*) są nie akceptowane przez ślimaka i potencjalnie mogą być wykorzystane w celu ograniczania żerowania ślimaków na roślinach uprawnych. Stwierdzono też, że siewki rdestu ptasiego (*Polygonum aviculare*) mogą być wykorzystane jako alternatywne pożywienie ślimaków.