Article

Connection between Nutrient Content and Resistance to Selected Pests Analyzed in *Brassicaceae* Hybrids

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Abstract: Rapeseed (*Brassica napus* L. ssp. *oleifera* Metzg) is attacked by a wide range of pests. Breeding of resistant cultivars is one of the best methods of protecting crops against insects, and non-cultivated species of *Brassicaceae* can be used as resistance donors. In this study, we investigated the relationship between nutrient content and resistance to two commonly occurring pests (*Delia radicum* and *Brevicoryne brassicae*) in 12 *Brassicaceae* hybrid combinations. The experiment was conducted at Poznań University of Life Sciences Experimental Station located in Dłóń. The assessment was carried out for three years (2017, 2018, and 2019) on randomly chosen individuals in two growth stages: vegetative stage and flowering stage. The resistance to *B. brassicae* and *D. radicum* was observed in all three years, however, macronutrient and micronutrient contents as well as total nitrogen, total sulfur, and sulfur sulfate were observed only in 2019. Statistically significant negative association between Ca and resistance to *B. brassicae* and *D. radicum* was discovered. Additionally, positive association between Cu and resistance to *B. brassicae* was observed. The importance of selected mineral elements in plant resistance is widely discussed.

Keywords: rapeseed; *Delia radicum*; *Brevicoryne brassicae*; nutrients; hybrids

Citation: Szwarc, J.; Niemann, J.; Bocianowski, J.; Jakubus, M.; Mrówczyński, M. Connection between Nutrient Content and Resistance to Selected Pests Analyzed in *Brassicaceae* Hybrids. *Agriculture* **2021**, *11*, 94. <https://doi.org/10.3390/agriculture11020094>

Received: 20 November 2020

Accepted: 20 January 2021

Published: 23 January 2021

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1. Introduction

Rapeseed (*Brassica napus* L. ssp. *oleifera* Metzg) is a major crop plant and one of the most important oil sources in the world [1,2]. Currently, rapeseed growers face numerous problems that limit plant productivity, quality, and seed yield such as climate changes affecting the growth condition or increased pest infestation [3]. Rapeseed may be attacked by a large number of specialist insects. Cabbage aphid (*Brevicoryne brassicae* L.) (Homoptera: Aphididae) and cabbage root fly (*Delia radicum* L.) (Diptera: Anthomyiidae) are economically important pests in many regions of the world including Poland.

D. radicum is a pest originating from Europe, present across the entire Northern Hemisphere [4]. Larvae of *D. radicum* damage rapeseed plants by chewing mines in the roots, occasionally penetrating the stem and the head of the plant. Larvae feeding results in delayed growth, wilting, and stunting. Heavy infestation may result in reduced seed number or even plant death [5].

B. brassicae is a highly specialized pest, attacking only the *Brassicaceae* family. Insect is present in most temperate regions. Up to 15–20 generations can be completed during the growing season, depending on weather conditions [4,6]. Rapid development rate often results in severe infestation, leading to the death of young seedlings and wilting of adult plants. Other symptoms include yellowing and curling of the leaves, deformation of heads,

and reduced yield, often followed by contamination caused by the presence of honeydew and dead aphids [4,6]. *B. brassicae* is also an important vector of virus diseases [7].

Breeding resistant cultivars is one of the best methods of protecting rapeseed against dangerous insects. It is also considered as the most economical and ecological friendly approach of reducing the number of many pests [8]. One of the limiting factors of this method is an insufficient number of resistance genes within the cultivated species of *Brassicaceae* family. On the other hand, non-cultivated species of *Brassicaceae* can be an interesting source of genetic variability, and serve as a donor of resistance to insects. Interspecific crosses between chosen wild genotypes and rapeseed plants may allow the transfer of useful agronomical traits such as resistance to certain pests or diseases. This is why our research team's work in the Department of Genetics and Plant Breeding (Poznań University of Life Sciences) focuses on obtaining and studying hybrid *Brassicaceae* plants, especially analyzing the presence of resistance to commonly occurring insect pests. Recently, we concentrated our efforts on investigating the relationship between the amount of selected elemental minerals in hybrid plants and their field resistance to insects. Nutrients, both macro- and micronutrients, are essential for plant development. Traditionally, three nutrients (i.e., N, P and K) have been considered to be the most crucial, as most crops have high requirements for them. Apart from the above-mentioned elemental minerals, S, Ca, Mg and Na also play a significant role in plant metabolism. Moreover, micronutrients are responsible for increasing both the quantity and quality of crop yield through their involvement in the metabolism of N, P, K, Ca, Mg, and S as well as crop responses to environmental stress conditions [9,10]. Aside from significant influence on yield amount, some mineral elements play a major role in plant response to herbivores. For example, calcium ions act as a second messenger in signal pathways after herbivore attack. They activate calcium-sensitive proteins and eventually lead to the formation of jasmonates and activation of defense genes [11].

Macro- and micronutrients are responsible for various functions as structural components in macromolecules [12]. First of all, a special role is assigned to macronutrients because they are generally required at the concentration of greater than 0.1% of dry matter. In the case of the *Brassicaceae* family, the significance of sulfur is underlined. Sulfur, besides nitrogen, is involved in synthesizing plant defense compounds such as glucosinolates. These compounds are hydrolyzed as a result of plant cell damage under the influence of the enzyme myrosinase. Together with the enzyme glucosinolases, which breaks them down, they constitute the two-component defense system of plants against pathogens and pests [11,13,14]. Aside from its great effect on glucosinolate content, nitrogen also affects the potential attractiveness of a plant to pests as it plays a major role in determining plant architecture and quality [15]. A similar real meaning may be attached to many compounds that contain S because they have metabolic, structural, and regulatory functions and are ubiquitous [16,17].

As mentioned earlier, besides macronutrients, micronutrients are a necessary requirement by plants, although in smaller amounts—less than 0.01% of dry matter. Regardless, micronutrients play an important role in the growth and development of plants. Some metals (i.e., Cu and Zn) and their compounds show remarkable bactericidal and fungicidal activity [12].

Considering the significant roles of macro- and micronutrients in plant physiology, the potential influence of element concentrations in plant tissues of *Brassicaceae* hybrids on resistance to chosen pests was investigated as a main aim of the study. In detail, research was connected to the plants' response to two different insects: *Delia radicum* (Cabbage root fly) and *Brevicoryne brassicae* (Cabbage aphid).

2. Materials and Methods

2.1. Plant Material

Twelve hybrid combinations (F₅–F₇) were used as research material (Table 1). All genotypes were obtained at the Department of Genetics and Plant Breeding, Poznań University of Life Sciences (PULS) with the use of *in vitro* technique described by Niemann et al. [18].

Table 1. List of hybrid genotypes used in the study.

No. of Line	Cross Combination
H1	<i>B. napus</i> cv. Lisek × <i>B. fruticulosa</i> —PI 649097
H2	<i>B. napus</i> cv. Jet Neuf × <i>B. carinata</i> —PI 649094
H3	<i>B. napus</i> cv. Lisek × <i>B. carinata</i> cv. Dodola
H4	<i>B. napus</i> cv. Lisek × <i>B. fruticulosa</i> —PI 649099
H5	<i>B. napus</i> cv. Jet Neuf × <i>B. rapa</i> ssp. <i>pekinensis</i> 08 007569
H6	<i>B. napus</i> cv. Lisek × <i>B. tournefortii</i>
H7	<i>B. napus</i> cv. Lisek × <i>B. oleracea</i> var. <i>alboglabra</i>
H8	<i>B. napus</i> cv. Górczański × <i>B. rapa</i> ssp. <i>pekinensis</i> 08 007574
H9	<i>B. napus</i> cv. Jet Neuf × <i>B. carinata</i> —PI 649096
H10	<i>B. napus</i> cv. Californium × <i>B. fruticulosa</i> —PI 649097
H11	<i>B. napus</i> cv. Jet Neuf × <i>S. alba</i> cv. <i>Bamberka</i>
H12	<i>B. napus</i> cv. Californium × <i>B. rapa</i> ssp. <i>pekinensis</i> 08 007574

2.2. Field Conditions

The experiment was conducted at the Poznań University of Life Sciences Experimental Station located in Dłóń (51°41′23.835″ N 17°4′1.414″ E), on a typical heavy soil of III quality class. The experiment was carried out in a randomized complete block design with three replications. For plant analysis, we chose 10 plants per hybrid in each replication in each year of study. Agricultural practices were optimal for local agroecological conditions. Wheat (*Triticum aestivum* L.) was used as a forecrop in three preceding years. The pre-sowing fertilization consisted of ammonium phosphate (200 kg ha^{−1}) and ammonium nitrate (100 kg ha^{−1}). During spring, four more fertilizers were used: Saletrosan 26 (300 kg ha^{−1}), ammonium nitrate (200 kg ha^{−1}), and ADOB Bor, containing boron and nitrogen, which was also used as an autumn treatment. One fungicide—Caryx 240SL (1 L ha^{−1}) was used during plant growth.

2.3. Assessment of Pest Resistance

Resistance to two insects, *Delia radicum* and *Brevicoryne brassicae*, was assessed during field trials. The assessment of general damage caused by *D. radicum* and *B. brassicae* infestation was performed in 2017, 2018, and 2019. The evaluation of direct damage on roots for *D. radicum*, and the percentage of infested plants by *B. brassicae* was executed according to the European and Mediterranean Plant Protection Organization (EPPO) standards [19] on randomly chosen individuals (Figures 1 and 2). For every genotype, ten plants were assessed in each replication. The severity of insect damage and plant infestation were evaluated at physiological maturity on a one to nine scoring scale, used commonly by the Research Center for Cultivar Testing in Poland, which corresponds to the International Union for the Protection of New Varieties of Plants [20] system of assessment. According to this scale, for *D. radicum*, a score of nine means no visible damage on plants (highly resistant), a score of five means that lesions are visible on up to 25% of the roots and surface of the leaves (moderately resistant), and a score of one means a completely damaged plant (fully susceptible). For *B. brassicae*, a score of nine means no insects present on the plant (highly resistant), a score of five means small and medium sized colonies on the plant (moderately resistant), and a score of one means that the plant is completely covered by aphid colonies (fully susceptible). The average values from ten plants were calculated for each replication, which allowed us to obtain quantitative traits data with the normal distributions.

Figure 1. *B. brassicae* infestation on the leaf of the *Brassicaceae* hybrid.

Figure 2. Damage caused by *D. radicum* on the root of the *Brassicaceae* hybrid.

2.4. Analysis of Plant Materials

Whole plants were collected from the field. For every genotype, ten randomly chosen individuals were assessed. The plant material was collected at two dates corresponding to

the vegetation phases: the BBCH 11–14 rosette phase (vegetative stage) and the flowering phase BBCH 63–65 (flowering stage). Plant material, consisting of fresh leaves and stems, was dried at 60 °C, ground and ashed in a furnace at 450 °C for 6 h. The ash was dissolved in 5 mL of 6 mol·dm^{−3} HCl [21] and diluted to a constant volume with distilled water. The obtained extracts were analyzed to assess macronutrient (K, Ca, Mg and Na) and micronutrient (Cu, Zn, Mn, Fe and Ni) contents using atomic absorption spectroscopy (ASA) in a Varian Spectra AA 220 FS apparatus. For total nitrogen (N_{tot}) and phosphorus content (P_{tot}), plant samples were analyzed separately and two different methods were performed. Total phosphorus content was measured colorimetrically by the ammonium methanadinate method and total nitrogen was analyzed by the Kjeldahl method [21]. Total sulfur (S_{tot}) and sulfur sulfate (S-SO₄) were determined by turbidimetric methods by Butters and Chenery [22] and Bardsley and Lancaster [23], respectively. All the assays identifying the amounts of mineral elements (nutrients) were performed in three replications of the same biological sample. The macronutrient and micronutrient contents as well as total nitrogen, total sulfur, and sulfur sulfate were observed only in 2019 in two growth stages: vegetative stage and flowering stage.

2.5. Statistical Analysis

The normality of the distributions of the studied elements (N_{tot}, P_{tot}, S_{tot}, S-SO₄, K, Ca, Na, Mg, Cu, Zn, Mn, Ni, and Fe) were tested using Shapiro–Wilk’s normality test [24]. Multivariate analysis of variance (MANOVA) was performed. Next, two-way analysis of variance (ANOVA) was carried out to determine the effects of hybrids and growth stages as well as hybrid × growth stage interaction on the variability of N_{tot}, P_{tot}, S_{tot}, S-SO₄, K, Ca, Na, Mg, Cu, Zn, Mn, Ni, and Fe. The mean values and standard deviations of mineral elements were calculated. The Fisher’s least significant differences (LSDs) were calculated for individual minerals and on this basis, homogeneous groups were determined. The relationships between analyzed nutrients were assessed on the basis of Pearson’s correlation for growth stages independently. Relationships of observed mineral elements were presented in heatmaps. Results were also analyzed using multivariate methods. The canonical variate analysis was applied in order to present multi-trait assessment of similarity of tested hybrids in a lower number of dimensions with the least possible loss of information [25]. This makes it possible to illustrate variation in species in growth stages of all observed mineral elements in the graphic form. The Mahalanobis distance [26] was suggested as a measure of “poly-trait” hybrids similarity [27], whose significance was verified by means of critical value D_α , called “the least significant distance” [28]. Mahalanobis distances were calculated for hybrids, independently for growth stages. The associations between observed mineral elements, resistance to *Brevicoryne brassicae*, and resistance to *Delia radicum* were estimated using regression analysis in both growth stages independently in each year of study. The mineral element observations were treated as independent variables and considered in individual models. In all analyses, we used the critical significance levels equal to 0.05, 0.01, and 0.001, resulting from a Bonferroni correction. All the analyses were conducted using the GenStat 18th edition statistical software package.

3. Results

3.1. Macro- and Micronutrient Analysis

The results of the MANOVA performed indicated that all the hybrids ($F_{11;48} = 64.99$; $p < 0.0001$), growth stages ($F_{1;48} = 3112.04$; $p < 0.0001$), and hybrid × growth stage interaction ($F_{11;48} = 45.84$; $p < 0.0001$) were significantly different with regard to all of the 13 quantitative mineral elements. Analysis of variance indicated that the main effects of hybrids as well as hybrid × growth stage interaction were significant for all the nutrients of study (Table 2). The main effects of growth stages were significant for all the nutrients except K (Table 2). The mean values and standard deviations for the observed mineral

elements indicated a high variability among the tested hybrids for which significant differences were found in growth stages of all the analyzed nutrients (Table 3).

Table 2. Mean squares form two-way analysis of variance for analyzed nutrients.

Source of Variation	Hybrid	Growth Stage	Hybrid × Growth Stage	Residual
d.f.	11	1	11	48
Ptot	0.007 ***	0.122 ***	0.004 ***	0.001
S-SO ₄	2.257 ***	24.743 ***	1.314 ***	0.073
Ntot	37.148 ***	435.563 ***	31.765 ***	0.8435
Stot	1.567 ***	158.554 ***	0.498 ***	0.1296
K	17.082 ***	0.381	29.106 ***	0.383
Ca	30.857 ***	587.549 ***	21.277 ***	0.128
Na	0.002 ***	0.004 ***	0.003 ***	0.001
Mg	0.120 ***	3.472 ***	0.082 ***	0.001
Cu	0.405 ***	4.661 ***	0.605 ***	0.014
Zn	2744.726 ***	9092.936 ***	2807.555 ***	4.711
Mn	1473.782 ***	12,554.258 ***	463.411 ***	5.329
Ni	0.886 ***	8.487 ***	0.514 ***	0.069
Fe	78,015.46 ***	643,093.3 ***	75,226.66 ***	26.87

*** $p < 0.001$.

The correlation analysis within nutritional elements indicated statistically significant correlation coefficients for Zn and Fe, Mn and Fe, S-SO₄ and Cu, Ntot and Mn, K and Na, Ca and Mg, Ca and Cu, Ca and Fe, and Na and Mg in both growth stages (Table 4, Figures 3 and 4). Additionally, in the vegetation stage, we observed significant correlation between Ptot and Stot, Ptot and Zn, Stot and S-SO₄, Stot and Zn, Stot and Fe, K and Mn, Ca and Zn, Ca and Ni, Na and Ni, Mg and Ni, Cu and Zn, Cu and Fe as well as Zn and Mn (Table 2, Figure 3). In the flowering stage, the pairs Ptot-K, Ptot-Mn, S-SO₄-K, S-SO₄-Ca, S-SO₄-Mg, S-SO₄-Mn, Ntot-Ca, Ntot-Fe, Stot-K, K-Ca, K-Mg, K-Zn, Ca-Na, Ca-Mn, Na-Fe, Mg-Cu, Mg-Mn, Mg-Fe, Cu-Mn and Cu-Ni were significantly correlated (Table 4, Figure 4).

Individual nutrients are of different importance and have a different share in the joint multivariate variation. A study on the multivariate variation for hybrids also includes the identification of the most important mineral elements in the multivariate variation of hybrids. Analysis of canonical variables is a statistical tool making it possible to solve the problem of multivariate relationships [29–32]. Figures 5 and 6 show the variability of the 13 nutrients of 12 studied rapeseed hybrids in the growth stages of the first two canonical variables in the first and second growth stage, respectively. In the graph, the coordinates of the point for particular hybrids are the values for the first and second canonical variables, respectively. The first two canonical variables accounted for 95.93% and 78.11% of the total multivariate variability between the individual hybrids in the vegetative stage and flowering stage, respectively (Table 5, Figures 5 and 6). The most significant, negative, linear relationship between the first canonical variables was found for Zn and Fe in the vegetative stage (Table 5); the second canonical variable in the vegetative stage was significantly positively correlated with Ni and negatively correlated with Ca, Mg, and Mn (Table 5). In the flowering stage, the most significant, positive, linear relationship between the first canonical variables was found for Ntot, Ca, Mg, Mn, and Fe (Table 5). The second canonical variable in the flowering stage was significantly positively correlated with S-SO₄, K, Ca, and Mg (Table 5). The greatest variation of all 13 nutrients jointly (measured Mahalanobis distances) was found for hybrids H3 and H9 (in vegetative stage) and H4 and H6 (in flowering stage), the Mahalanobis distance between them amounted to 232.13 and 86.35, respectively (Table 6). The greatest similarity was found for hybrids H8 and H11 (13.69) in the vegetative stage as well as between H1 and H5 (8.45) in the flowering stage (Table 6). The correlation coefficient between Mahalanobis distances in both terms was equal to -0.1606 and was not significant ($p = 0.1977$).

Table 3. Mean values and standard deviations (s.d.) for observed nutrients (N = 90).

Nutrient		Ptot				S-SO ₄				Ntot				Stot				K			
Growth stage		I		II		I		II		I		II		I		II		I		II	
Hybrid	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	
H1	0.772	0.011	0.827	0.008	1.943	0.198	3.493	0.359	44.1	0.254	38.96	2.290	2.633	0.239	5.918	0.081	15.58	0.975	17.43	0.074	
H2	0.789	0.028	0.890	0.012	2.449	0.125	2.226	0.514	45.08	0.676	42.7	0.790	2.917	0.441	6.039	0.224	21.87	0.748	14.09	0.572	
H3	0.783	0.032	0.871	0.041	2.395	0.231	2.989	0.268	43.95	0.370	41.24	0.769	2.609	0.053	6.037	0.298	21.36	0.612	18.15	0.099	
H4	0.777	0.024	0.872	0.048	2.973	0.188	2.822	0.042	41.81	0.498	39.42	0.232	2.891	0.149	5.764	0.705	17.14	0.492	14.33	0.092	
H5	0.764	0.002	0.837	0.022	2.266	0.274	2.977	0.129	40.11	0.422	40.88	0.199	2.28	0.336	6.077	0.300	18.66	1.117	19.26	0.307	
H6	0.773	0.016	0.847	0.018	1.951	0.603	3.244	0.160	40.87	0.896	32.88	0.073	2.4	0.265	6.439	0.235	19.76	0.588	21.35	0.375	
H7	0.704	0.015	0.792	0.018	2.105	0.209	3.352	0.285	48.71	1.027	37.88	0.089	3.15	0.079	5.607	0.097	17.41	0.420	20.81	0.133	
H8	0.725	0.021	0.929	0.017	2.531	0.543	4.843	0.298	44.88	0.540	43.61	0.295	3.577	0.239	6.28	0.552	22.33	0.446	18.71	0.718	
H9	0.774	0.010	0.830	0.037	2.053	0.074	2.678	0.101	44.75	2.167	30.35	0.106	3.703	0.076	6.326	0.669	22.74	0.210	17.78	0.034	
H10	0.758	0.050	0.880	0.069	3.003	0.110	5.885	0.265	41.92	1.390	33.69	0.481	4.113	0.100	6.428	0.935	19.29	1.646	20.83	0.296	
H11	0.719	0.004	0.803	0.009	2.747	0.091	3.967	0.152	42.35	1.821	37.91	0.109	4.19	0.108	6.423	0.290	18.17	0.486	21.88	0.563	
H12	0.727	0.046	0.717	0.023	2.23	0.156	4.241	0.102	41.96	0.362	41.95	0.165	4.363	0.140	7.103	0.109	17.25	0.608	25.19	0.472	
LSD _{0.05}	0.044		0.054		0.479		0.433		1.775		1.281		0.367		0.772		1.328		0.643		

Nutrient		Ca				Na				Mg				Cu				Zn			
Growth Stage		I		II		I		II		I		II		I		II		I		II	
Hybrid	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	
H1	4.4	0.020	10.11	0.266	0.179	0.002	0.187	0.006	0.506	0.012	1.059	0.053	4.207	0.090	4.703	0.172	75.92	4.783	23.82	0.862	
H2	5.93	0.216	10.46	0.423	0.250	0.011	0.183	0.001	0.726	0.031	0.9	0.033	4.233	0.061	4.003	0.057	62.89	6.362	21.79	0.932	
H3	6.52	0.106	12.71	0.361	0.209	0.008	0.216	0.010	0.738	0.020	1.243	0.006	4.373	0.070	4.86	0.020	165.8	4.323	17.51	0.868	
H4	6.8	0.146	11.75	0.147	0.236	0.012	0.255	0.019	0.764	0.041	1.211	0.042	3.507	0.031	4.04	0.115	42.64	0.839	32.09	1.330	
H5	10.72	0.450	11.86	0.352	0.239	0.008	0.212	0.003	0.957	0.058	1.125	0.053	3.593	0.147	4.757	0.078	25.26	3.092	23.24	1.161	
H6	6.33	0.203	9.12	0.128	0.262	0.030	0.229	0.009	0.726	0.008	0.871	0.025	4.59	0.281	4.653	0.012	21.8	0.559	17.75	0.661	
H7	7.75	0.087	18.19	0.530	0.258	0.013	0.211	0.004	0.948	0.003	1.405	0.017	3.867	0.058	4.737	0.023	21.93	0.344	19.25	0.849	
H8	8	0.431	16.03	0.690	0.260	0.009	0.234	0.030	0.678	0.012	1.33	0.044	3.46	0.072	5.143	0.021	22.1	1.623	20.31	2.150	
H9	7.41	0.001	6.75	0.276	0.228	0.036	0.192	0.007	0.646	0.022	0.952	0.040	4.227	0.227	3.767	0.111	26.54	2.076	16.54	0.113	
H10	7.6	0.123	17.52	0.254	0.201	0.001	0.218	0.003	0.654	0.028	1.472	0.007	3.4	0.080	4.633	0.256	18.27	0.211	22.6	1.074	
H11	8.73	0.300	12.63	0.860	0.232	0.017	0.165	0.003	0.751	0.013	1.014	0.044	3.95	0.070	4.03	0.010	20.55	0.017	19.2	0.421	
H12	7.37	0.205	19	0.481	0.187	0.005	0.265	0.013	0.774	0.032	1.555	0.041	4.113	0.101	4.3	0.100	18.9	0.850	18.77	0.106	
LSD _{0.05}	0.396		0.7553		0.027		0.020		0.047		0.063		0.218		0.183		4.876		1.728		

Table 3. Cont.

Nutrient	Mn				Ni				Fe			
	I		II		I		II		I		II	
Growth Stage	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.
Hybrid												
H1	24.59	0.516	58.39	1.917	3.8	0.300	2.39	0.135	332.7	4.770	84.5	1.061
H2	66.98	1.497	71.38	1.610	2.833	0.058	1.75	0.128	278.9	2.962	61	3.555
H3	63.91	2.506	80.7	0.232	3	0.529	3.407	0.546	960.6	15.677	85.8	1.486
H4	35.5	4.358	55.44	3.878	3.067	0.351	1.74	0.386	215.3	4.051	119.6	4.845
H5	30.67	1.723	65.33	1.541	2.033	0.208	2.057	0.029	173.4	9.900	80.7	1.061
H6	25.95	3.765	53.17	1.478	2.4	0.100	2.08	0.115	211.7	2.235	45.4	0.567
H7	56.17	4.792	92.07	1.390	2.167	0.252	2.14	0.036	179.9	0.740	89.4	2.597
H8	42.95	0.197	96.3	2.239	3.2	0.100	2.357	0.418	179.4	0.952	83.7	1.765
H9	46.55	2.470	33.33	1.558	3.133	0.306	2.303	0.218	144.8	10.980	48.1	1.465
H10	47.96	1.422	88.05	2.410	2.433	0.208	1.827	0.292	147.7	2.743	98.7	0.756
H11	25.75	0.230	54.59	1.480	3.1	0.200	1.99	0.070	156.5	0.367	72.6	0.113
H12	15.63	2.680	50.78	1.153	2.8	0.100	1.687	0.197	243	7.238	86.2	3.540
LSD _{0.05}	4.439		3.25		0.438		0.448		11.7		3.953	

Table 4. The correlation coefficients matrix for nutrients analyzed in the vegetative stage (above diagonal) and flowering stage (below diagonal) (N = 90).

Nutrient	Ptot	S-SO ₄	Ntot	Stot	K	Ca	Na	Mg	Cu	Zn	Mn	Ni	Fe
Ptot	1	0.02	−0.25	−0.46 **	0.17	−0.32	−0.12	−0.3	0.24	0.40 *	0.2	0.21	0.3
S-SO ₄	0.09	1	−0.22	0.37 *	−0.01	0.18	0.01	0	−0.52 **	−0.05	0.13	0.01	−0.07
Ntot	0.04	−0.05	1	0.06	0.14	−0.27	0.18	0	0.06	0.14	0.54 ***	0.12	0.08
Stot	−0.32	0.3	−0.08	1	0.04	0.18	−0.25	−0.15	−0.26	−0.42 *	−0.17	0.14	−0.34 *
K	−0.53 ***	0.53 ***	−0.15	0.52 **	1	0.1	0.36 *	−0.08	0.1	0.16	0.57 ***	−0.02	0.17
Ca	−0.22	0.65 ***	0.37 *	0.18	0.55 ***	1	0.28	0.69 ***	−0.51 **	−0.44 **	−0.12	−0.53 ***	−0.33 *
Na	0.02	0.19	0.19	0.29	0.23	0.44 **	1	0.43 **	−0.01	−0.28	0.29	−0.41 *	−0.27
Mg	−0.14	0.63 ***	0.29	0.15	0.46 **	0.91 ***	0.57 ***	1	−0.22	−0.21	0.09	−0.72 ***	−0.1
Cu	0.29	0.40 *	0.31	−0.02	0.22	0.41 *	0.24	0.39 *	1	0.40 *	0.02	0.12	0.41 *
Zn	0.19	−0.05	0.24	−0.27	−0.52 **	0	0.26	0.08	−0.09	1	0.46 **	0.32	0.96 ***
Mn	0.41 *	0.44 **	0.42 *	−0.22	−0.03	0.62 ***	0.09	0.50 **	0.71 ***	0.03	1	−0.13	0.40 *
Ni	0.21	−0.16	0.07	−0.06	−0.09	−0.16	−0.11	−0.06	0.39 *	−0.32	0.2	1	0.2
Fe	0.1	0.33	0.39 *	−0.21	−0.11	0.56 ***	0.42 **	0.68 ***	0.21	0.71 ***	0.40 *	−0.06	1

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; green—correlation coefficients significant in both vegetative and flowering stages with the same sign; red—correlation coefficients significant in both vegetative and flowering stages with the opposite sign.

Table 5. Correlation coefficients between the first two canonical variables and nutrients in both terms (N = 90).

Nutrient	Vegetative Stage		Flowering Stage	
	First Canonical Variable	Second Canonical Variable	First Canonical Variable	Second Canonical Variable
Ptot	−0.3721	0.2918	0.2488	−0.2435
S-SO ₄	0.067	−0.3284	0.1973	0.7333 **
Ntot	−0.1418	−0.2435	0.6071 *	−0.0484
Stot	0.4106	0.1114	−0.4367	0.4807
K	−0.1285	−0.0412	−0.2577	0.8729 ***
Ca	0.3175	−0.6427 *	0.6113 *	0.7067 *
Na	0.2642	−0.4646	0.4248	0.1661
Mg	0.0271	−0.8156 **	0.6718 *	0.6005 *
Cu	−0.3758	0.5651	0.3847	0.5392
Zn	−0.9693 ***	0.1223	0.5569	−0.5692
Mn	−0.448	−0.4653	0.6824 *	0.4192
Ni	−0.1824	0.6783 *	0.0558	0.1199
Fe	−0.9912 **	0.0967	0.8769 ***	0.0079
Percentage variation	91.56%	4.37%	57.32%	20.79%

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.**Table 6.** The Mahalanobis distances between the studied hybrids in vegetative stage (above diagonal) and flowering stage (below diagonal) (N = 90).

Hybrid	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12
H1	0	33.13	173.38	37.9	61.39	49.54	57.3	58.35	66.71	65.05	61.69	46
H2	28.37	0	181.88	24.48	44.88	45.17	31.63	44.59	55.27	50.09	52.16	43.64
H3	28.22	37.42	0	195.66	208.44	215.72	200.46	219.72	232.13	227.49	223.93	206.38
H4	46.06	49.29	45.79	0	28.93	34.68	26.07	31.64	46.06	35.54	36.56	32.1
H5	8.45	29.46	25.08	47.36	0	38.97	27.91	28.78	44.35	32.72	29.85	35.9
H6	42.63	54.68	61.9	86.35	43.74	0	46.13	21.59	22.85	28.45	20.74	19.21
H7	38.99	45.99	17.49	50.39	34.73	68.96	0	36.7	50	37.85	41.9	41.95
H8	32.6	39.78	16.13	51.15	28.48	63	17.05	0	19.21	14.56	13.69	27.49
H9	43.2	50.36	64.55	79.56	47.5	25.5	72.46	68.37	0	20.43	20.43	34.58
H10	35.84	48.17	28.36	53.36	33.72	60.5	21.3	24.01	63.09	0	17.19	33.23
H11	29.65	44.47	47.94	71.58	29.34	23.12	52.85	47.68	32.93	43.25	0	23.02
H12	36.7	56.64	41.49	57.93	34.64	54.85	41.83	40.9	57.9	33.69	39.15	0

Figure 3. Heatmap for linear Pearson's correlation coefficients between observed nutrients in the vegetative stage ($r_{cr} = 0.36$).

Figure 4. Heatmap for linear Pearson correlation coefficients between observed nutrients in the flowering stage ($r_{cr} = 0.36$).

Figure 5. Distribution of 12 hybrids in the space of the first two canonical variables in the vegetative stage (N = 90).

Figure 6. Distribution of 12 hybrids in the space of the first two canonical variables in the vegetative stage (N = 90).

3.2. Resistance to Pests

The resistance to *B. brassicae* and *D. radicum* of the hybrids was widely described by Niemann et al. [33]. In our previous research, we assessed the resistance to *B. brassicae* and *D. radicum* within 53 rapeseed cultivars and 31 interspecific hybrids including twelve genotypes investigated in this study. Generally, the resistance to insects of studied hybrid plants varied between genotypes and years. For *B. brassicae*, mean resistance values for hybrids in 2017, 2018, and 2019 were 8.766, 6.250, and 7.610, respectively. The highest level of resistance (9.000) was observed in 2017 for three genotypes: *B. napus* cv. Jet Neuf $\times$ *B. carinata*-PI 649094, *B. napus* cv. Jet Neuf $\times$ *B. carinata*-PI 649096, and *B. napus* cv. Jet Neuf $\times$ *S. alba* cv. Bamberka, while the lowest level of resistance (3.000) was reported in 2018 for *B. napus* cv. Górczański $\times$ *B. rapa* ssp. *pekinensis* 08.007574. For *D. radicum*, mean resistance values for hybrids in 2017, 2018, and 2019 were 6.542, 6.611, and 6.864, respectively. The highest level of resistance (8.400) was noticed in 2017 for *B. napus* cv. Lisek $\times$ *B. fruticulosa*-PI649099 and in 2019 for *B. napus* cv. Lisek $\times$ *B. tournefortii*. Meanwhile, the lowest level of resistance (2.300) was observed in 2017 for one hybrid—*B. napus* cv. Lisek $\times$ *B. fruticulosa*-PI649097.

3.3. Associations between Pest Resistance and Nutrient Content

The associations between analyzed nutrients, resistance to *B. brassicae*, and resistance to *D. radicum* are presented in the Tables 7 and 8, respectively. We observed a statistically significant negative (-0.2101) association between Ca in the vegetative stage and resistance to *B. brassicae* in 2019 ($p = 0.033$). Percentage of variation of resistance to *B. brassicae* was accounted by a Ca variation equal to 31.8% (Table 7). Additionally, a positive (0.817) association between Cu in the vegetative stage and resistance to *B. brassicae* was observed in 2019 ($p = 0.036$). Percentage of variation of resistance to *B. brassicae* accounted by Cu variation was equal to 30.6% (Table 7). The resistance to *D. radicum* in 2019 was statistically significantly negatively (-0.6) associated with Ca in the vegetative stage and $p = 0.014$. Percentage of variation of resistance to *D. radicum* accounted by Ca variation was equal to 41.5% (Table 8).

Table 7. The associations between the analyzed nutrients and resistance to *Brevicoryne brassicae* (N = 90).

Nutrient	Growth Stage	Estimate	<i>p</i> Values	Percentage of Variation Accounted	Estimate	<i>p</i> Values	Percentage of Variation Accounted	Estimate	<i>p</i> Values	Percentage of Variation Accounted
		2017			2018			2019		
P _{tot}	vegetative stage	−0.76	0.726	10.5	−0.7	0.959	22.3	9	0.107	16.2
	flowering stage	0.2	0.865		−12.23	0.069		−2.15	0.503	
S-SO ₄	vegetative stage	0.225	0.161	4.3	−0.32	0.759	23.2	−0.076	0.87	6.2
	flowering stage	−0.0422	0.482		−0.228	0.541		−0.073	0.663	
N _{tot}	vegetative stage	0.0173	0.51	20.1	0.045	0.784	3.1	−0.0086	0.906	31.8
	flowering stage	0.0099	0.502		−0.1571	0.064		−0.0487	0.219	
S _{tot}	vegetative stage	0.0949	0.25	9.6	0.227	0.666	11.7	0.085	0.717	8.5
	flowering stage	0.002	0.992		−0.089	0.928		0.37	0.39	
K	vegetative stage	0.0431	0.081	1.3	−0.09	0.582	12.6	0.0289	0.694	7.4
	flowering stage	−0.0137	0.482		0.094	0.438		−0.0019	0.972	
Ca	vegetative stage	0.0522	0.172	0.7	0.115	0.642	45.77	−0.2101	0.033	89.07
	flowering stage	0.0009	0.955		−0.0039	0.969		−0.0274	0.533	
Na	vegetative stage	2.11	0.324	15.1	−6.7	0.62	11.9	−7.63	0.185	2.8
	flowering stage	−0.87	0.678		−10.6	0.409		0.62	0.915	
Mg	vegetative stage	0.5	0.31	11.9	3.34	0.271	12.6	−1.74	0.191	8.1
	flowering stage	0	1		0.01	0.997		−0.254	0.735	
Cu	vegetative stage	−0.102	0.515	15.1	0.694	0.473	11.7	0.817	0.036	30.6
	flowering stage	−0.216	0.116		−1.246	0.148		−0.519	0.178	
Zn	vegetative stage	0.0001	0.94	11.9	−0.0015	0.87	12.6	0.0048	0.215	6.4
	flowering stage	−0.0037	0.804		−0.0685	0.451		−0.0283	0.484	
Mn	vegetative stage	0.0052	0.146	11.9	0.0111	0.633	12.6	0.0065	0.526	2.8
	flowering stage	0	1		−0.017	0.388		−0.0094	0.279	
Ni	vegetative stage	−0.011	0.929	11.9	−1.083	0.139	12.6	0.16	0.64	7.4
	flowering stage	−0.009	0.949		−0.269	0.745		0.106	0.773	
Fe	vegetative stage	0.00003	0.926	11.9	−0.0001	0.937	12.6	0.00094	0.201	7.4
	flowering stage	−0.00001	0.997		−0.0075	0.686		−0.0055	0.501	
Total percent of variation accounted by all 13 nutrients				67.81	45.77				89.07	

Table 8. The associations between analyzed nutrients and resistance to *Delia radicum* (N = 90).

Nutrient	Growth Stage	Estimate	<i>p</i> Values	Percentage of Variation Accounted	Estimate	<i>p</i> Values	Percentage of Variation Accounted	Estimate	<i>p</i> Values	Percentage of Variation Accounted
		2017			2018			2019		
P _{tot}	vegetative stage	−1.3	0.937	12.1	13	0.341	2.1	5.6	0.715	1.6
	flowering stage	6.11	0.505		7.8	0.292		2.77	0.74	
S-SO ₄	vegetative stage	1.84	0.144	21.6	−0.94	0.382	11.4	−0.5	0.677	41.5
	flowering stage	−0.298	0.529		−0.534	0.151		0.122	0.777	
N _{tot}	vegetative stage	0.016	0.94	25.7	0.069	0.687	10.8	0.189	0.302	11
	flowering stage	0.01	0.935		−0.0964	0.304		−0.078	0.454	
S _{tot}	vegetative stage	0.323	0.628	17.9	−0.806	0.119	11.8	0.184	0.76	0.4
	flowering stage	−0.12	0.922		−1.554	0.106		0.28	0.808	
K	vegetative stage	0.347	0.072	2.0	0.209	0.206	3.8	0.098	0.602	0.4
	flowering stage	−0.093	0.548		−0.151	0.22		−0.016	0.907	
Ca	vegetative stage	0.471	0.111	8.4	0.28	0.264	5.2	−0.6	0.014	0.4
	flowering stage	0.015	0.903		−0.176	0.065		0.024	0.831	
Na	vegetative stage	30.2	0.053	5.0	14.7	0.283	1.9	−3.1	0.841	85.73
	flowering stage	12.5	0.446		−18.3	0.157		20.7	0.144	
Mg	vegetative stage	6.17	0.095	89.17	2.18	0.499	91.73	−4.84	0.155	85.73
	flowering stage	0.33	0.879		−2.42	0.147		1.11	0.565	
Cu	vegetative stage	−1.06	0.384	8.4	−0.021	0.983	3.8	1.06	0.331	0.4
	flowering stage	−1.17	0.295		0.052	0.956		0.12	0.909	
Zn	vegetative stage	−0.0045	0.694	5.0	0.0029	0.756	5.2	0.0053	0.603	0.4
	flowering stage	0.013	0.913		−0.0377	0.693		−0.042	0.693	
Mn	vegetative stage	0.0374	0.187	5.0	0.0266	0.258	5.2	0.0152	0.567	0.4
	flowering stage	0.0041	0.871		−0.0041	0.844		0.0027	0.906	
Ni	vegetative stage	−1.12	0.237	5.0	−0.528	0.508	5.2	0.731	0.4	0.4
	flowering stage	−0.39	0.713		0.99	0.234		0.46	0.626	
Fe	vegetative stage	−0.0004	0.847	89.17	0.0001	0.963	91.73	0.0013	0.511	85.73
	flowering stage	0.0033	0.89		−0.0196	0.297		−0.0001	0.996	
Total percent of variation accounted by all 13 nutrients				89.17	91.73				85.73	

4. Discussion

The pathogens and insects are responsible for the reduced crop yield, so presently, a variety of fungicides and pesticides are applied in agriculture. Taking into account the current requirements of sustainable agriculture with less consumption of agrochemicals, more attention is paid to the natural defense mechanisms of plants against pests and pathogens. It can be assumed that plants with the proper concentration of nutrients in plant tissues will resist pests more, and the research carried out confirmed such a statement indirectly.

B. napus plants are rich in sulfur as it is necessary for plant development. Sulfur is used to synthesize glucosinolate and phytoalexins, which play an important role in plant defense systems [34]. Marazzi and Stadler [35] found a correlation between sulfur plant nutrition and *D. radicum* development by testing *B. napus* plants with three different levels of sulfur supply: no sulfur added, normal dose of sulfur, and double dose of sulfur. The conducted experiment showed that larval survival to pupation, adult emergence, and adult fresh weight was significantly greater when *B. napus* plants received normal or double the normal dose of sulfur. In contrast, our research showed no connection between sulfur content in plants and resistance to *D. radicum*. As stated before, *B. brassicae* is a highly specialized pest. The insect adapts so well that it takes advantage of the plants' own defense system by using glucosinolates produced by the host as protection against predatory species [36]. As glucosinolates do not harm the insect, it would be expected that an increased amount of sulfur and nitrogen in the plant, which are compounds of this toxic chemical, will not impact the number of *B. brassicae* individuals that damage the plants. This assumption corresponds well with our results.

Based on our study, no relation was discovered between nitrogen content and resistance to *D. radicum* and *B. brassicae*. Furthermore, in 2011, Mochiah et al. [37] studied how insect population in *Brassica oleracea* is affected by the use of different soil fertilizers (i.e., NPK and poultry manure). Conducted field trials showed that both organic and synthetic nutrients could increase the population of some pests on cabbage, but the same effect was not observed for *B. brassicae*. However, experiments performed by Mutiga et al. [38] showed clearly that increasing the quantity of nitrogen applied in the soil led to an increase in *B. brassicae* infestation on collard (*Brassica oleracea* var. *acephala*). This phenomena might be explained by enhanced amino acid synthesis caused by increased N level, as it changes the nutritional quality of floem sap; though it is crucial to emphasize that is it the balance and composition of different amino acids that plays the major role [14,38]. Furthermore, a study from Chesnais et al. [39] proved that an increase in N level may indeed positively impact *B. brassicae* size and fecundity, though using excessive nitrogen treatments may limit aphid fertility.

The group of macronutrients also includes calcium, although it is not as required by *Brassica* plants as sulfur or nitrogen. However, the role it plays in plant cells is significant. Calcium is an essential second messenger in many pathways, especially in plant response to pathogen attacks [40,41]. Lecourieux et al. [40] made an exhaustive review of various calcium compounds and their functions in plant organisms and pointed to the importance of calmodulin and calmodulin-binding proteins in plant defense against fungal pathogenic. Based on this, one can risk a theory that the compounds with the participation of calcium including the aforementioned calmodulin can also determine the resistance of plants to pests. The obtained findings from our own research confirm this in some way, as we discovered a significant association between the resistance to *D. radicum* and Ca, and between the resistance to *B. brassicae* and Ca in the last year of our study.

In this study, a positive relationship was shown between the amount of copper in plant cells and resistance to *B. brassicae* (in the third year of the study) This result should be considered interesting and intriguing, more so because copper is attributed with a strong fungicidal and bactericidal effect [12]. Considering the fact that when Cu is present in a low concentration, it acts as a cofactor for metallo-proteins and enzymes, whereas at higher concentrations, it performs as an antimicrobial agent against pathogens. In this aspect, it

would have to be considered whether the Cu level in the plants was sufficiently adequate to induce a pest resistance effect.

Pest management of oilseed rape in Europe relies mostly on chemical treatments. Unfortunately, pesticides are often used as a preventive measure in many cases without precise recognition of insects in the field [42]. Such a demeanor leads to the over-use of broad spectrum chemicals and results in pesticide resistance and pest resurgence [38]. Moreover, uncaredful application, especially during the flowering period, may have harmful effects on beneficial insects such as bees [43]. Therefore, it is particularly relevant to develop a consistent, environmentally friendly approach to limit the pests' population. Considering all the presented results, it cannot be confirmed that adjusted plant nutrition is a sufficient replacement for using pesticides, though it should be considered as part of a non-chemical strategy against dangerous pests, along with biological control and breeding resistant cultivars.

5. Conclusions

Despite the fact that the presented outcomes showed correlation between elemental accumulation expressed by the concentration of nutrients and plant resistance to pests only for Ca and Cu, research in this direction should be continued, as they fit into the philosophy of sustainable agriculture. The role of adjusted plant nutrition in plant resistance should be further analyzed.

Author Contributions: Conceptualization, J.N., J.S., and J.B.; Methodology, J.S. and J.N.; Software, J.B.; Validation, J.S., J.N., and J.B.; Formal analysis, J.N., M.J., and M.M.; Investigation, J.S., J.N., M.J., and M.M.; Data curation, J.S., J.N., and J.B.; Writing—original draft preparation, J.S.; Writing—review and editing, J.N., J.B., and M.J.; Visualization, J.B.; Supervision, J.S., J.N., and J.B.; Project administration, J.N.; Funding acquisition, J.N. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Polish Ministry of Agriculture and Rural Development, project number 54.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. The Food and Agriculture Organization (FAO): FAOSTAT. Production/Yield Quantities of Rapeseed in World. 2020. Available online: <http://www.fao.org/faostat/en/#data/QC/visualize> (accessed on 23 October 2020).
2. United States Department of Agriculture. Oilseeds: World Markets and Trade. 2020. Available online: <https://apps.fas.usda.gov/psdonline/circulars/oilseeds.pdf> (accessed on 23 October 2020).
3. Corlouer, E.; Gauffreteau, A.; Bouchet, A.S.; Bissuel-Bélaygue, C.; Nesi, N.; Laperche, A. Envirotypes Based on Seed Yield Limiting Factors Allow to Tackle G×E Interactions. *Agronomy* **2019**, *9*, 798. [CrossRef]
4. Capinera, J.L. *Handbook of Vegetable Pests*; Academic Press Elsevier: London, UK, 2020.
5. Mason, P.G.; Huber, J.T. *Biological Control Programmes in Canada, 1981–2000*; CABI: Wallingford, UK, 2002.
6. Hines, R.L.; Hutchison, W.D. *Cabbage Aphids. VegEdge, Vegetable IPM Resource for the Midwest*; University of Minnesota: Minneapolis, MN, USA, 2013; Available online: <https://www.vegedge.umn.edu/pest-profiles/pests/cabbage-aphids> (accessed on 23 October 2020).
7. Garbe, V.; Gladders, P.; Lane, A. Report of the Concerted Action. In Proceedings of the Research for the Adaption of Oilseed Crops to the New Requirements of the Common Agricultural Policy: Crop Competitiveness, Seed Quality, Environment, Integrated Control in Oilseed Crops IOBC/wprs Bulletin, Einsiedeln, Switzerland, 2–4 November 2000; Volume 23, pp. 173–178.
8. Arora, R.; Sandhu, S. (Eds.) *Breeding Insect Resistant Crops for Sustainable Agriculture*; Springer: Singapore, 2017.
9. Jakubus, M.; Bakinowska, E. Varied macronutrient uptake by plants as an effect of different fertilisation schemes evaluated by PCA. *Acta Agric. Scand. Sect. B Soil Plant Sci.* **2020**, *70*, 56–68. [CrossRef]
10. Jakubus, M.; Graczyk, M. Microelement variability in plants as an effect of sewage sludge compost application assessed by different statistical methods. *Agronomy* **2020**, *10*, 642. [CrossRef]
11. Fürstenberg-Hägg, J.; Zagrobelny, M.; Bak, S. Plant Defense against Insect Herbivores. *Int. J. Mol. Sci.* **2013**, *14*, 10242–10297. [CrossRef] [PubMed]

12. Rai, M.; Ingle, A.P.; Pandit, R.; Paralikar, P.; Shende, S.; Gupta, I.; Biswas, J.K.; Silverio da Silva, S. Copper and copper nanoparticles: Role in management of insect-pests and pathogenic microbes. *Nanotechnol. Rev.* **2018**, *7*, 303–315. [\[CrossRef\]](#)
13. Jakubus, M. *Sulphur in Environment*; Poznań University of Agriculture: Wojska Polskiego, Poznań, 2006; Volume 48. (In Polish)
14. Cole, R.A. The relative importance of glucosinolates and amino acids to the development of two aphid pests *Brevicoryne brassicae* and *Myzus persicae* on wild and cultivated brassica species. *Entomol. Exp. Appl.* **1997**, *85*, 121–133. [\[CrossRef\]](#)
15. Rusch, A.; Valantin-Morison, M.; Sarthou, J.P.; Roger-Estrade, J. Integrating Crop and Landscape Management into New Crop Protection Strategies to Enhance Biological Control of Oilseed Rape Insect Pests. In *Biocontrol-Based Integrated Management of Oilseed Rape Pest*; Williams, I.H., Ed.; Springer Science+Business Media B.V.: Berlin/Heidelberg, Germany, 2010; pp. 415–448.
16. Jakubus, M.; Graczyk, M. Quantitative changes in sulphur fractions during co-composting of pine bark with green plant material. *Pol. J. Environ. Stud.* **2019**, *28*, 1–12. [\[CrossRef\]](#)
17. Kopriva, S.; Calderwood, A.; Weckopp, S.C.; Koprivova, A. Plant sulphur and big data. *Plant Sci.* **2015**, *241*, 1. [\[CrossRef\]](#)
18. Niemann, J.; Wojciechowski, A.; Janowicz, J. Broadening the variability of quality traits in rapeseed through interspecific hybridization with an application of immature embryo culture. *Biotechnol. J. Biotechnol. Comput. Biol. Bionanotechnol.* **2012**, *93*, 109–115. [\[CrossRef\]](#)
19. Anon. *Guidelines on Pest Risk Analysis. No. 3. Pest Risk Assessment, Scheme*; Bulletin OEPP/EPPO Bulletin: Paris, France, 1997; Volume 27, pp. 281–305.
20. UPOV: International Union for the Protection of New Varieties of Plants. *Guidance on Certain Physiological Characteristics*; Document TGP/12; UPOV: Geneva, Switzerland, 2012.
21. Ostrowska, A.; Gawlinski, S.; Szczubialka, Z. *Metody Analizy i Oceny Wlasciowosci Gleb i Roslin: Katalog*; Dział Wydawnictw IOŚ: Warszawa, Poland, 1991; p. 334.
22. Butters, B.; Chenery, E.M. A rapid method for the determination of total sulphur in soils and plants. *Analyst* **1959**, *84*, 239–245. [\[CrossRef\]](#)
23. Bardsley, C.E.; Lancaster, J.D. Determination of reserve sulfur and soluble sulfates in soils 1. *Soil Sci. Soc. Am. J.* **1960**, *24*, 265–268. [\[CrossRef\]](#)
24. Shapiro, S.S.; Wilk, M.B. An analysis of variance test for normality (complete samples). *Biometrika* **1965**, *52*, 591–611. [\[CrossRef\]](#)
25. Rencher, A.C. Interpretation of canonical discriminant functions, canonical variates, and principal components. *Am. Stat.* **1992**, *46*, 217–225.
26. Mahalanobis, P.C. On the generalized distance in statistics. *Proc. Natl. Acad. Sci. India A* **1936**, *12*, 49–55.
27. Seidler-Łożykowska, K.; Bocianowski, J. Evaluation of variability of morphological nutrients of selected caraway (*Carum carvi* L.) genotypes. *Ind. Crops Prod.* **2012**, *35*, 140–145. [\[CrossRef\]](#)
28. Camussi, A.; Ottaviano, E.; Caliński, T.; Kaczmarek, Z. Genetic distances based on quantitative nutrients. *Genetics* **1985**, *111*, 945–962. [\[CrossRef\]](#)
29. Seidler-Łożykowska, K.; Bocianowski, J.; Król, D. The evaluation of the variability of morphological and chemical nutrients of the selected lemon balm (*Melissa officinalis* L.) genotypes. *Ind. Crop. Prod.* **2013**, *49*, 515–520. [\[CrossRef\]](#)
30. Lahuta, L.B.; Ciak, M.; Rybiński, W.; Bocianowski, J.; Börner, A. Diversity of the composition and content of soluble carbohydrates in seeds of the genus *Vicia* (Leguminosae). *Genet. Resour. Crop Evol.* **2018**, *65*, 541–554. [\[CrossRef\]](#)
31. Wrońska-Pilarek, D.; Szkudlarz, P.; Bocianowski, J. Systematic importance of morphological features of pollen grains of species from *Erica* (Ericaceae) genus. *PLoS ONE* **2018**, *13*, e0204557.
32. Bocianowski, J.; Majchrzak, L. Analysis of effects of cover crop and tillage method combinations on the phenotypic nutrients of spring wheat (*Triticum aestivum* L.) using multivariate methods. *Appl. Ecol. Environ. Res.* **2019**, *17*, 15267–15276. [\[CrossRef\]](#)
33. Niemann, J.; Szwarc, J.; Bocianowski, J.; Weigt, D.; Mrówczyński, M. In-field screening for host plant resistance to *Delia radicum* and *Brevicoryne brassicae* within selected rapeseed cultivars and new interspecific hybrids. *Open Life Sci.* **2020**, *15*, 711–720. [\[CrossRef\]](#)
34. Ahuja, I.; Rohloff, J.; Bones, A.M. Defence mechanisms of Brassicaceae: Implications for plant-insect interactions and potential for integrated pest management. A review. *Agron. Sustain. Dev.* **2010**, *30*, 311–348. [\[CrossRef\]](#)
35. Marazzi, C.; Städler, E. Influence of sulphur plant nutrition on oviposition and larval performance of the cabbage root fly. *Agric. For. Entomol.* **2005**, *7*, 277–282. [\[CrossRef\]](#)
36. Kuśnierczyk, A.; Winge, P.E.R.; Jørstad, T.S.; Troczyńska, J.; Rossiter, J.T.; Bones, A.M. Towards global understanding of plant defence against aphids—timing and dynamics of early Arabidopsis defence responses to cabbage aphid (*Brevicoryne brassicae*) attack. *Plant Cell Environ.* **2008**, *31*, 1097–1115. [\[CrossRef\]](#)
37. Mochiah, M.B.; Baidoo, P.K.; Owusu-Akyaw, M. Influence of different nutrient applications on insect populations and damage to cabbage. *J. Appl. Biosci.* **2011**, *38*, 2564–2572.
38. Mutiga, S.K.; Gohole, L.S.; Auma, E.O. Effects of integrating companion cropping and nitrogen application on the performance and infestation of collards by *Brevicoryne brassicae*. *Entomol. Exp. Appl.* **2010**, *134*, 234–244. [\[CrossRef\]](#)
39. Chesnais, Q.; Couty, A.; Catterou, M.; Ameline, A. Cascading effects of N input on tritrophic (plant–aphid–parasitoid) interactions. *Ecol. Evol.* **2016**, *6*, 7882–7891. [\[CrossRef\]](#)
40. Lecourieux, D.; Ranjeva, R.; Pugin, A. Calcium in plant defence—signaling pathways. *N. Phytol.* **2006**, *171*, 249–269. [\[CrossRef\]](#)
41. Ma, Y.; Gerald, A.; Berkowitz, S. Biotic Stress Signalling: Ca²⁺ Mediated Pathogen Defence Programmes. In *Plant Stress Physiology*, 2nd ed.; Shabala, S., Ed.; CAB International: Wallingford, UK, 2017.

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42. Williams, I.H. Advances in Insect Pest Management of Oilseed Rape in Europe. In *Insect Pest Management: Field and Protected Crops*, 1st ed.; Horowitz, A.R., Ishaaya, I., Eds.; Springer: Berlin, Germany, 2004; pp. 181–208.
 43. Mänd, M.; Williams, I.H.; Viik, E.; Karise, R. Oilseed Rape, Bees and Integrated Pest Management. In *Biocontrol-Based Integrated Management of Oilseed Rape Pest*; Williams, I.H., Ed.; Springer Science+Business Media B.V.: Berlin/Heidelberg, Germany, 2010; pp. 357–380.