Brown Bead of Broccoli. II. Relationships of the Physiological Disorder with Nutritional and Meteorological Variables

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Abstract. In order to investigate their relationships with brown bead, a data set composed of 48 variables characterizing the developmental rate, climate, and nutrients in the soil and in the tissues of heads of broccoli (*Brassica oleracea* L., Italica group) was collected from 328 plots (41 experimental fields over 3 years $\times$ 4 N fertilization levels $\times$ 2 blocks). The four N treatments were 85–0–0, 85–54–0, 85–54–54, and 85–54–108, the first number indicating the N level (kg-ha⁻¹) applied before planting; the second, N level applied 5 weeks after planting; and the last, N level applied 7 weeks after planting. Broccoli plants were either direct-seeded (26 experimental fields) or transplanted (15 experimental fields). Whether direct-seeded or transplanted, fast-developing broccoli plants showed a lower incidence of brown bead. More particularly, heads of transplanted broccoli plants experiencing warmer temperatures had a lower brown bead incidence and severity. A regular supply of water decreased the incidence and severity of the physiological disorder in both direct-seeded and transplanted broccoli plants. Low levels of Ca and high levels of Mg and K in mature broccoli head tissues were associated with a higher incidence of brown bead. Multiple-regression models were developed to predict the percentage of broccoli heads with brown bead for direct-seeded plants ($R^2 = 0.76$; $n = 104$), and for transplanted plants ($R^2 = 0.69$; $n = 44$). For direct-seeded broccoli, solar radiation between the button stage (head diameter of 2.5 cm) and maturity (head diameter of 10 cm), as well as soil and tissue Mg content, were among the first variables to enter the regression models. In general, more solar radiation and less precipitation translated into more heads showing brown bead symptoms. For transplanted broccoli plants, the minimum temperature from the button stage to maturity was a key variable in the prediction of the percentage of heads with brown bead and the corresponding index of severity.

Brown bead is a physiological disorder that was first observed in broccoli (*Brassica oleracea* L., Italica group) in the 1970s during

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a production boom in California. Subsequently, brown bead was responsible for the abandonment of substantial acreage and loss of product quality in this state (Steta, 1987). Restriction of the production to areas and periods more suitable for the climatic requirements of broccoli as well as use of more resistant varieties considerably reduced the occurrence of the disorder.

Broccoli is one of Canada's 10 major imported fresh vegetables, with 76,000 t of broccoli imported in 1998 (Agriculture and Agri-Food Canada, 1999). In 1999, 19,000 t of broccoli were grown on 1500 ha of land in the province of Quebec, which represented 57% of the Canadian production. Presently, brown bead is responsible for up to one-third of the field rejects. Up to now, little work has been done on understanding the causes leading to brown bead. Under field conditions, Steta

(1987) reported that N levels of 150, 300, and 600 kg-ha⁻¹ had no significant effect on the percentage of broccoli buds showing brown bead. Low Ca levels associated with rapid growth were thought to be related to brown bead (Flint, 1985), although this relationship was not clearly demonstrated (Steta, 1987). In contrast, Pascual et al. (1996) reported that broccoli heads with brown bead had higher Ca content and lower K than healthy ones.

Broccoli grows best in the temperature range of 16 to 18 °C, with a minimum of 4 °C and a maximum of 24 °C (Maynard and Hochmuth, 1997). High temperature has often been associated with the appearance of brown bead. In a controlled environment, temperatures above 30 °C have induced a greater percentage of heads with brown bead than under temperatures in the range of 15 to 25 °C (Steta, 1987). In another study, the incidence of brown bead increased exponentially with increasing maximum temperature (22 to 38 °C) during the 5-d period preceding head maturity (Lopez-Galarza et al., 1993). Steta (1987) observed that high relative humidity (90%) reduced brown bead incidence, even under high temperature (35 °C). Brown bead may also be related to the presence of ethylene, involved in abscission and senescence induction in various fruits and vegetables (Sexton et al., 1985). Plants treated with ethephon had 66% of the heads with brown bead, a significantly higher value compared with 0.3% for nontreated plants (Steta, 1987).

The objectives of this study were to investigate, under field conditions, the relationships between the incidence and severity of brown bead, and variables characterizing the development rate, climate, and mineral nutrition of broccoli heads.

Materials and Methods

We refer to Jenni et al. (2001) for a complete description of the field layout, planting dates, and soil condition. In this 3-year study, data were collected for 50 variables (Table 1) from 328 plots (41 experimental fields $\times$ 4 N fertilization levels $\times$ 2 blocks). Plant density was calculated in each plot by counting the number of broccoli plants in two beds containing two rows over a 4-m distance. The incidence of brown bead was expressed as the percentage of plants presenting the symptoms (BBP, in %). A brown bead index (BBI, from 0 = no symptoms to 8 = very severe symptoms) expressed the severity of the symptoms and was evaluated on each head at commercial maturity according to Jenni et al. (2001). Button stage was defined as the time when 50% of the heads were 2.5 cm or more in diameter, and commercial maturity as the time when at least 50% of the heads reached 10 cm in diameter.

Tissue and soil analyses. When broccoli plants reached the button stage in an experimental field, leaves were randomly sampled from each plot before 10:00 AM and two drops of sap were extracted from the ribs (Hochmuth, 1994). Three measurements of tissue NO₃-N were made using a Nitrate kit (Geneq, Montréal). Tissue analysis was performed on

a sample of four broccoli heads randomly harvested at commercial maturity from the two central beds of each plot. Head florets were cut at 0.5 cm and dried at 21 °C for 48 h. Determination of P, K, Ca, Mg, B, Cu, Fe, and Mn in head tissue was performed by inductively coupled plasma optical emission spectrometry (ICP-OES), and determination of N was made by segmented-flow analysis (SFA) using Isaac-type digestion (Isaac and Johnson, 1976). Sulfur and molybdenum were measured by ICP-OES in a nitric acid digestion for S (Havlin and Soltampour, 1980) and after dry ashing for Mo (Gupta, 1998). For soil analysis purposes, four subsamples of soil were randomly taken from each plot in each experimental field. The four subsamples were homogenized to constitute one soil sample on which pH and organic matter were determined (McKeague, 1978). Phosphorus, K, Ca, Mg, B, Cu, Fe, and Mn were determined in these soil samples by ICP-OES using Mehlich 3 extraction (Mehlich, 1984), whereas nitrate content was measured by SFA using 2 M potassium chloride extraction (Bran Luebbe Analyzing Technologies, 1989).

Meteorological data. ACR-10X datalogger (Campbell Scientific, Edmonton, Alta.) located on the farm site recorded readings every 10 mn, and averaged or totaled the recorded data every hour. The following meteorological variables were recorded: average air temperature (°C) at 1.2 m, average relative humidity (%), Vaisala 50Y temperature and relative humidity probe), total solar radiation (kJ·m⁻², LI-COR Silicon Pyranometer), total rain (mm, Texas Electronics Tipping Bucket Rain Gauge), and average wind speed (m·s⁻¹, RM Young Wind Monitor). In addition, two TruChek rain gauges (Geneq) were placed in each experimental field to estimate the cumulative height of irrigation water (mm). For each type of meteorological variable, an average (temperature, relative humidity, wind) or sum (solar radiation, precipitation including irrigation) was calculated over the three following periods: 1 week following the button stage, from the button stage to commercial maturity, and 5 d preceding commercial maturity.

Development rates. For each experimental field, development rates were defined as $(1/t) \times 100$, where t (in days) is the time required to complete a development process from planting or direct seeding to the button stage (TXBU) or to commercial maturity (TXMA).

Statistical analyses. We refer to Jenni et al. (2001) for the preliminary treatment of the data as well as the detection of outliers. Data from the 3 years were combined in order to allow the examination of trends and relationships over the entire range of the environmental variables during the course of the experiment. This was consistent with the random nature of the year factor in the analyses of variance performed by Jenni et al. (2001). To investigate the relationships between the BBP and BBI variables and the other quantitative variables in the data set, Spearman's rank-based correlation coefficients were analyzed using SAS procedure CORR (SAS Institute,

Table 1. Codes for variables.

Code	Variables	Units
DENS	Population density	No-plants/m ²
TXBU	Development rate from seeding or planting to the button	d ⁻¹
TXMA	Development rate from seeding or planting to maturity	d ⁻¹
NCKB	N leaf sap content at the button stage	ppm
PTIS	Head tissue P content at maturity	%
KTIS	Head tissue K content at maturity	mg·kg ⁻¹
CATIS	Head tissue Ca content at maturity	mg·kg ⁻¹
MGTIS	Head tissue Mg content at maturity	mg·kg ⁻¹
BTIS	Head tissue B content at maturity	mg·kg ⁻¹
CUTIS	Head tissue Cu content at maturity	mg·kg ⁻¹
FETIS	Head tissue Fe content at maturity	mg·kg ⁻¹
MNTIS	Head tissue Mn content at maturity	mg·kg ⁻¹
MOTIS	Head tissue Mo content at maturity	mg·kg ⁻¹
STIS	Head tissue S content at maturity	mg·kg ⁻¹
NSOILB	Soil N at button stage	mg·kg ⁻¹
PHMA	Soil water pH at broccoli maturity	---
OMMA	Soil organic matter content at broccoli maturity	%
PSOIL	Soil P at broccoli maturity	mg·kg ⁻¹
KSOIL	Soil K at broccoli maturity	mg·kg ⁻¹
CASOIL	Soil Ca at broccoli maturity	mg·kg ⁻¹
MGSOIL	Soil Mg at broccoli maturity	mg·kg ⁻¹
BSOIL	Soil B at broccoli maturity	mg·kg ⁻¹
CUSOIL	Soil Cu at broccoli maturity	mg·kg ⁻¹
FESOIL	Soil Fe at broccoli maturity	mg·kg ⁻¹
MNSOIL	Soil Mn at broccoli maturity	mg·kg ⁻¹
NSOILM	Soil N at broccoli maturity	mg·kg ⁻¹
MINBU	Average minimum temperature during 1 week after button stage	°C
MINBM	Average minimum temperature from button stage to maturity	°C
MINMA	Average minimum temperature during 5 d before maturity	°C
MAXBU	Average maximum temperature during 1 week after button stage	°C
MAXBM	Average maximum temperature from button stage to maturity	°C
MAXMA	Average maximum temperature during 5 d before maturity	°C
RIBU	Rain and irrigation during 1 week after button stage	mm
RIBM	Rain and irrigation from button stage to maturity	mm
RIMA	Rain and irrigation during 5 d before maturity	mm
RADBU	Solar radiation during 1 week after button stage	kJ·m ⁻²
RADBM	Solar radiation from button stage to maturity	kJ·m ⁻²
RADMA	Solar radiation during 5 d before maturity	kJ·m ⁻²
RHBU	Average relative humidity during 1 week after button stage	%
RHBM	Average relative humidity from button stage to maturity	%
RHMA	Average relative humidity during 5 d before maturity	%
WBU	Average wind speed during 1 week after button stage	m·s ⁻¹
WBM	Average wind speed from button stage to maturity	m·s ⁻¹
WMA	Average wind speed during 5 d before maturity	m·s ⁻¹
BBP	Percentage of heads with brown bead	%
BBI	Index of severity on heads showing brown bead symptoms	0–8
KRTIS	K:(Ca+Mg) ratio (head tissues)	---
KRSOIL	K:(Ca+Mg) ratio (soil)	---
CARTIS	Ca:(K+Mg) ratio (head tissues)	---
CARSOIL	Ca:(K+Mg) ratio (soil)	---

1997). Multiple-regression models for the prediction of the BBP and BBI variables were generated using the stepwise selection of variables in the SAS procedure REG (SAS Institute, 1997). The significance level for a regressor to remain in the model was set at 0.1.

Results

Spearman's rank-based correlation analysis. Many correlation coefficients between the variables of incidence (BBP) and severity (BBI), and the quantitative variables characterizing climate and nutrients in soil and tissue of broccoli heads were of different sign and magnitude for seeded and transplanted broccoli plants and for the N fertilization levels considered separately (Tables 2 and 3). Therefore, seeded and transplanted broccoli plants did not appear to respond similarly to their environment regarding brown bead.

Variables expressing incidence and severity of brown bead in transplanted broccoli plants were often correlated with meteorological variables (Tables 2 and 3). Brown bead incidence and severity decreased in transplanted plants as minimum and maximum temperature increased. With all fertilization levels considered together, BBP was negatively correlated with MINBU ($r = -0.300^{**}$), MINBM ($r = -0.334^{**}$), and MAXBU ($r = -0.528^{**}$), and BBI was negatively correlated with MINMA ($r = -0.346^{**}$), MAXBM ($r = -0.291^{**}$), and MAXMA ($r = -0.392^{**}$).

For all N fertilization levels considered together, the percentage of heads with brown bead symptoms was often negatively correlated with the amounts of water supplied to the plots, particularly during periods close to maturity ($r = -0.313^{**}$ for the direct-seeded plants and $r = -0.414^{**}$ for the transplants; Table 2). The severity index increased as wa-

Table 2. Spearman rank-based correlations between the percentage of broccoli heads with brown bead symptoms and quantitative variables related to climate and nutrient content of soil and tissue for seeded vs. transplanted broccoli, the four levels of N fertilization considered separately and all together^a.

N fertilization	Seeded broccoli plants					Transplanted broccoli plants				
	All levels	85–0–0	85–54–0	85–54–54	85–54–108	All levels	85–0–0	85–54–0	85–54–54	85–54–108
Mean	0.277	0.3226	0.2867	0.2381	0.262	0.322	0.354	0.3251	0.3076	0.2994
SE	0.141	0.139	0.1446	0.1319	0.1418	0.159	0.1833	0.1439	0.1606	0.1576
n	104	26	26	26	26	60	15	15	15	15
DENS	0.022	0.062	–0.006	0.202	–0.065	0.103	0.048	0.014	0.275	0.022
TXBU	0.025	0.077	–0.060	0.141	–0.024	–0.310	–0.198	–0.257	–0.120	–0.478
TXMA	–0.305**	–0.337	–0.329	–0.172	–0.323	–0.542**	–0.618*	–0.564*	–0.475	–0.551*
NCKB	–0.022	0.246	0.080	0.282	–0.105	0.190	0.086	0.395	0.490	0.057
PTIS	0.294**	0.236	0.365	0.180	0.393	–0.148	–0.036	–0.068	–0.125	–0.352
KTIS	0.512**	0.471*	0.529**	0.479*	0.569**	0.066	0.129	0.154	0.100	–0.086
CATIS	–0.218*	–0.364	–0.277	–0.106	–0.251	0.149	0.054	0.025	0.057	0.393
MGTIS	0.497**	0.351	0.450*	0.629**	0.515**	0.403**	0.229	0.289	0.582*	0.597*
BTIS	0.179	0.100	0.283	0.314	0.113	0.236	0.335	0.064	0.474	0.352
CUTIS	–0.333**	–0.251	–0.368	–0.400*	–0.254	–0.143	–0.553*	0.061	0.018	–0.263
FETIS	0.340**	0.303	0.579**	0.317	0.423	0.356**	0.318	0.064	0.711**	0.479
MNTIS	0.143	0.011	–0.035	0.448*	0.111	0.317**	0.359	0.475	0.413	0.055
MOTIS	–0.128	–0.253	–0.088	–0.171	–0.066	–0.079	–0.018	0.150	–0.157	–0.139
STIS	0.053	–0.036	–0.034	0.110	0.090	0.095	–0.004	0.016	0.032	0.345
NSOILB	0.128	0.208	0.436*	0.426*	0.299	–0.170	–0.249	–0.179	–0.033	0.071
PHMA	0.207*	0.223	0.177	0.141	0.227	0.387**	0.517*	0.443	0.358	0.241
OMMA	–0.102	–0.190	–0.033	–0.173	–0.007	–0.317**	–0.361	–0.404	–0.486	–0.114
PSOIL	0.336**	0.345	0.464*	0.362	0.266	–0.252	0.437	–0.039	–0.325	–0.368
KSOIL	0.158	0.106	0.119	0.242	0.256	–0.108	–0.343	–0.129	–0.014	0.236
CASOIL	0.053	–0.100	0.009	–0.030	0.281	0.017	0.136	–0.064	–0.168	0.143
MGSOIL	0.278**	0.357	0.209	0.329	0.383	–0.174	–0.329	–0.264	–0.064	0.157
BSOIL	0.255**	0.135	0.312	0.288	0.300	0.109	0.200	–0.054	0.090	0.168
CUSOIL	–0.067	–0.001	–0.091	–0.112	0.008	–0.268*	–0.520*	0.020	–0.361	–0.275
FESOIL	–0.023	0.025	0.074	–0.063	–0.067	–0.530**	–0.579*	–0.471	–0.596*	–0.479
MNSOIL	–0.009	0.041	–0.003	–0.041	–0.146	0.086	0.179	–0.068	0.102	0.109
NSOILM	0.135	0.106	0.414*	0.448*	0.505**	–0.151	–0.027	–0.272	0.032	0.175
MINBU	0.002	–0.045	–0.068	–0.049	0.177	–0.300**	–0.375	–0.549*	–0.399	–0.002
MINBM	0.013	–0.085	–0.043	–0.021	0.233	–0.334**	–0.444	–0.469	–0.406	–0.032
MINMA	0.021	–0.057	0.004	–0.011	0.198	–0.246	–0.206	–0.276	–0.281	–0.143
MAXBU	–0.083	–0.174	–0.127	–0.066	0.041	–0.528**	–0.551*	–0.805	–0.596*	–0.265
MAXBM	–0.106	–0.247	–0.102	–0.129	0.064	–0.049	–0.064	–0.211	–0.002	0.200
MAXMA	–0.092	–0.209	–0.093	–0.135	0.081	–0.003	0.009	0.025	0.086	0.086
RIBU	–0.338**	–0.374	–0.423*	–0.465*	–0.140	0.002	–0.086	0.143	–0.064	–0.166
RIBM	–0.168	–0.149	–0.301	–0.306	0.036	–0.312	–0.274	–0.166	–0.424	–0.581*
RIMA	–0.313**	–0.274	–0.425*	–0.353	–0.249	–0.414**	–0.530*	–0.372	–0.433	–0.478
RADBU	0.001	–0.060	0.004	–0.009	0.073	–0.014	–0.168	–0.068	–0.052	0.225
RADBM	0.298**	0.298	0.248	0.227	0.366	0.064	0.193	0.000	0.134	–0.014
RADMA	–0.319**	–0.473*	–0.315	–0.344	–0.131	0.268**	0.089	0.318	0.349	0.487
RHBU	0.298**	0.312	0.341	0.253	0.263	0.102	0.222	0.225	0.066	–0.190
RHBM	0.386**	0.410*	0.401*	0.330	0.388*	–0.290**	–0.179	–0.168	–0.360	–0.580*
RHMA	0.349**	0.347	0.373	0.312	0.319	–0.291**	–0.175	–0.193	–0.381	–0.540*
WBU	–0.091	–0.018	–0.199	–0.023	–0.074	0.323**	0.225	0.315	0.352	0.426
WBM	–0.057	0.034	–0.095	0.011	–0.084	0.115	–0.025	0.064	0.109	0.272
WMA	–0.212	–0.232	–0.180	–0.176	–0.214	–0.115	–0.229	–0.093	–0.199	–0.093
KRATIS	0.279*	0.413	0.348	0.152	0.342	–0.126	–0.029	–0.004	–0.054	–0.361
KRATIOSOIL	0.007	0.043	0.018	0.065	–0.123	–0.072	–0.282	–0.029	0.082	0.032
CRATIS	–0.320**	–0.401*	–0.378	–0.222	–0.381	0.142	0.029	0.050	0.064	0.354
CRATIOSOIL	–0.094	–0.253	–0.020	–0.104	–0.120	0.116	0.311	0.061	–0.014	0.032

^a. **Significant at $P \leq 0.05$ or 0.01, respectively.

ter supply decreased in the direct-seeded plots that received high levels of N (i.e., 85–54–104, $r = -0.489^*$). Therefore, a regular supply of water appeared to be an important factor to decrease the incidence and severity of brown bead.

For seeded broccoli plants, BBP and BBI tended to be positively correlated with tissue K, Mg, and Fe concentrations in the head and negatively correlated with tissue Ca and Cu concentrations (Tables 2 and 3). BBI tended to be negatively correlated with tissue Mo concentration.

Stepwise multiple regression. Regression models predicting the percentage of heads with brown bead symptoms were different for the direct-seeded and the transplanted broccoli plants (Tables 4 and 5). The variables

selected also differed among the models developed at each level of N fertilization. For all N fertilization levels considered together, 76% of the variation in the percentage of heads with brown bead symptoms was explained by 11 variables for direct-seeded broccoli plants ($n = 104$), and 69% by five variables for transplanted broccoli plants ($n = 44$).

The Mg concentration in the tissue of heads at maturity was an important variable closely related to both BBP and BBI for direct-seeded broccoli plants. For these plants, solar radiation between the button stage and maturity was important in predicting BBP as that climatic variable entered as first or second regressor in the models (Table 4). More solar radiation during this period, which probably

induced an increased transpiration, meant more heads with brown bead. The amounts of rain and irrigation water were also important in predicting BBP for direct-seeded broccoli, a greater supply of water resulting in fewer heads with brown bead.

For transplanted broccoli plants, minimum air temperature at time close to maturity was a key variable to predict BBP, that climatic variable being selected twice as the first regressor to enter the model (Table 4). The average minimum temperature between the button stage and maturity represented 49% of the variation of the BBP variable at the 85–54–0 N fertilization level, and 43% at the 85–54–54 N fertilization level. Otherwise, the variables associated with maximum air tempera-

Table 3. Spearman rank-based correlations between the index of brown bead severity and quantitative variables related to climate and nutrient content of soil and tissue for seeded vs. transplanted broccoli, the four levels of N fertilization considered separately and all together^a.

N fertilization	Seeded broccoli plants					Transplanted broccoli plants				
	All levels	85–0–0	85–54–0	85–54–54	85–54–108	All levels	85–0–0	85–54–0	85–54–54	85–54–108
Mean	3.281	3.667	3.349	3.123	2.985	3.944	4.187	3.908	3.836	3.845
SE	1.348	1.169	1.196	1.575	1.391	1.429	1.816	1.047	1.515	1.353
n	104	26	26	26	26	60	15	15	15	15
DENS	–0.043	–0.0732	–0.1789	0.1785	–0.031	0.316**	–0.1038	0.4129	0.2964	0.7276**
TXBU	0.158	0.0752	0.3216	0.1519	0.0842	–0.158	–0.0335	0.0475	–0.1844	–0.461
TXMA	–0.031	–0.1849	–0.0506	0.1351	–0.0381	0.148	0.3261	0.0688	0.3642	–0.096
NCKB	0.027	0.2161	0.04	0.2048	0.1474	–0.024	0.2357	0.1644	0.2288	–0.5786*
PTIS	0.029	–0.13	0.0371	–0.0722	0.2412	0.334**	0.2929	0.4767	0.2395	0.2377
KTIS	0.276**	0.1402	0.3154	0.1436	0.422*	0.259*	0.4893	0.2464	0.0089	0.0179
CATIS	0.076	–0.0988	0.1633	0.1026	0.0127	–0.263*	–0.3679	–0.4857	–0.1805	0.0107
MGTIS	0.468**	0.2774	0.349	0.5412**	0.6115**	–0.255*	–0.0964	–0.6107*	–0.4429	0.0143
BTIS	0.141	–0.0137	0.0802	0.2474	0.2862	–0.348**	–0.1377	–0.7979**	–0.3166	–0.288
CUTIS	–0.416**	–0.4512*	–0.3681	–0.2709	–0.5314**	–0.126	–0.2952	0.2931	–0.4429	–0.2075
FETIS	0.352**	0.2878	0.2777	0.5251**	0.4944*	0.005	0.2357	–0.1821	–0.0464	–0.2679
MNTIS	–0.115	–0.2659	–0.1738	0.0003	–0.0553	–0.077	–0.2181	–0.0161	0.0197	–0.0679
MOTIS	–0.37**	–0.4363*	–0.6389**	–0.3292	–0.2809	–0.083	–0.3714	0.2214	–0.2571	0.0786
STIS	0.283**	0.0441	0.3629	0.3847	0.2387	–0.275**	–0.3378	–0.4593	–0.3643	0.0894
NSOILB	0.006	0.0747	0.2153	0.1853	0.1102	–0.083	–0.2909	–0.0468	0.3879	0.0385
PHMA	–0.196*	–0.0787	–0.3607	–0.3217	–0.0602	0.002	–0.195	0.2786	–0.168	0.1752
OMMA	0.093	0.0537	0.305	0.048	0.1231	0.13	0.1393	–0.078	0.1	0.25
PSOIL	0.225*	0.4152*	–0.0646	0.2319	0.3516	0.338**	0.4122	0.1541	0.3071	0.3286
KSOIL	0.373**	0.3467	0.5284**	0.3101	0.381	–0.036	0.2714	–0.4607	–0.3786	0.1929
CASOIL	–0.057	–0.107	0.0219	–0.1056	–0.0301	0.388**	0.1714	0.7357**	0.1786	0.5393*
MGSOIL	0.334**	0.3235	0.3842	0.4564*	0.2482	–0.084	0.1893	–0.55*	–0.0036	–0.0071
BSOIL	0.157	0.0133	0.205	0.0425	0.4207*	0.109	0.1216	0.265	–0.0609	0.2433
CUSOIL	–0.227*	–0.1197	–0.2962	–0.416*	–0.0421	0.272*	0.2484	0.4879	0.3821	0.1376
FESOIL	0.028	0.0486	0.125	0.0899	–0.0995	0.01	0.1786	–0.2179	–0.1321	0.1179
MNSOIL	–0.243*	–0.0732	–0.3434	–0.3338	–0.3345	0.178	0.0464	0.0714	0.0643	0.3646
NSOILM	0.112	0.0103	0.3062	0.4923*	0.4114*	–0.132	–0.2914	–0.2724	0.2429	–0.0143
MINBU	0.066	–0.0024	0.0975	0.261	–0.0518	–0.089	–0.0521	–0.2119	–0.2621	0.1239
MINBM	0.001	–0.0896	0.0356	0.1515	–0.0262	–0.231	–0.3327	–0.2916	–0.3936	0.1055
MINMA	–0.054	–0.0448	–0.0577	0.0065	–0.0645	–0.346**	–0.4996	–0.2489	–0.5228*	–0.1468
MAXBU	0.064	–0.0301	0.0171	0.312	–0.0154	–0.022	0.186	–0.1914	–0.161	0.0125
MAXBM	0.027	–0.0763	0.0171	0.2405	–0.0284	–0.291**	–0.1503	–0.5528*	–0.5152*	–0.0912
MAXMA	–0.015	–0.0951	0.0132	0.0923	–0.0308	–0.392**	–0.3115	–0.6016*	–0.5891*	–0.1987
RIBU	–0.242*	–0.3711	–0.1213	–0.2743	–0.1593	0.025	–0.3023	0.1932	0.1342	0.0894
RIBM	–0.17	–0.198	0.0308	–0.2476	–0.2062	0.22	–0.0483	0.5523*	0.3378	0.109
RIMA	–0.233*	–0.0718	–0.1026	–0.2284	–0.4884*	0.113	–0.1038	0.4633	0.1288	0.0644
RADBU	0.071	0.1347	–0.0759	0.1173	0.1172	–0.196	–0.3327	–0.2451	–0.2039	0.1055
RADBM	0.223*	0.2738	0.3423	0.1097	0.2038	0.205	0.6225*	–0.0411	0.1753	–0.0948
RADMA	–0.181	–0.319	–0.1429	–0.0058	–0.2141	–0.125	–0.0894	–0.3309	–0.2755	0.195
RHBU	0.044	0.1216	–0.0144	–0.0527	0.0976	0.217	0.1395	0.288	0.3256	0.0268
RHBM	–0.031	0.0082	–0.1132	–0.1228	0.0869	0.19	0.0286	0.3882	0.3578	0.0304
RHMA	–0.05	–0.0267	–0.1026	–0.1217	0.0588	–0.046	–0.4329	0.3846	0.0894	–0.1127
WBU	–0.083	–0.0113	–0.1399	–0.2533	0.0955	–0.355**	–0.3435	–0.517*	–0.3757	–0.1449
WBM	–0.015	0.0482	0.0763	–0.1577	0.0038	–0.266*	–0.3005	–0.4347	–0.161	–0.0662
WMA	–0.144	–0.0684	–0.0404	–0.2387	–0.1936	0.252	0.0842	0.3241	0.5336*	0.1952
KRATIOTIS	–0.043	0.0653	–0.0506	–0.1179	0.0315	0.284**	0.4286	0.4929	0.1429	0.0179
KRATIOSOIL	0.274**	0.2595	0.3139	0.2615	0.2031	–0.266*	0.0893	–0.7286**	–0.425	–0.1036
CRATIOTIS	–0.003	–0.082	–0.011	0.050	–0.073	–0.269*	–0.429	–0.493	–0.146	0.018
CRATIOSOIL	–0.283**	–0.229	–0.357	–0.416**	–0.215	0.229	–0.129	0.754	0.021	0.329

*,**Significant at $P \leq 0.05$ or 0.01 , respectively.

ture did not appear in the models predicting BBP. Soil Ca was important in predicting BBP and BBI. That soil nutritional variable entered in the third position when all N fertilization levels were considered together, and in the first position at the 85–0–0 level for predicting BBP. Further, the soil Ca variable entered in the second position when all N fertilization levels were considered together and in the first position at the 85–54–108 level for predicting BBI (Tables 4 and 5).

Variables that did not intervene in any model included: Mn in broccoli head tissues (direct-seeded and transplanted plants) and soil (transplanted plant), soil K (direct-seeded or transplanted plants), and soil Fe (direct-seeded plants).

Discussion

Transplanted and direct-seeded broccoli plants did not respond in the same way to their surrounding environment with regards to brown bead. Seedlings growing in the restricted area of a multicell have an underdeveloped root system that tends to grow more in the upper horizons of the field soil. Transplanted plants are more sensitive to soil conditions in the plow layer (0–30 cm) than direct-seeded plants, which are more likely to exploit deeper soil horizons (30–60 cm) (NeSmith, 1999). Therefore, it is not surprising that field conditions promoting the fast development of a transplanted broccoli crop, which has to recover from the transplanting shock, would

tend to produce less brown bead. In contrast, high air temperature and fast development have often been associated with high incidence of brown bead (Hildebrand, 1994; Steta, 1987). In protected conditions, the average maximum temperature during the 5 d preceding commercial maturity was found to be highly correlated with the percentage of broccoli heads with brown bead (Lopez-Galarza et al., 1993). For instance, 10% of the heads had brown bead at 30°C compared with 50% at 36°C. In our study, the transplanted broccoli plants were produced early in the season and harvested before the warmest periods of the summer. We found a negative correlation between the development rate to commercial maturity and the percentage of heads with brown bead

Table 4. Stepwise multiple regressions in which the percentage of heads with brown bead symptoms is explained by climatic and nutritional variables for seeded vs. transplanted broccoli plants, the four levels of N fertilization considered separately and all together.

Variables	All N fertilization levels		85-0-0		85-54-0		85-54-54		85-54-108	
	Estimate	Order ²	Estimate	Order	Estimate	Order	Estimate	Order	Estimate	Order
<i>Seeded broccoli plants</i>										
INTERCEPT	-2.189***		0.7961*		-0.6212*		-0.9252*		-2.965***	
TXBU			-0.3530*	6						
TXMA									-0.7071***	4
NCKB	-0.0003022**	9								
KTIS			0.00001772*	5	0.00003239**	1			0.00000766**	10
CATIS									0.00002374***	6
MGTIS							0.0004353***	1		
BTIS									0.0030412**	7
FETIS									0.0008296**	12
MOMA			-0.04452*	4						
PSOIL	0.001080*	12								
MGSOIL	0.0003418***	7					0.0002476**	5	0.0006515***	1
BSOIL							0.1226***	4		
CUSOIL							-0.04801*	6		
MNSOIL	-0.003533***	5	-0.004199***	3					-0.003206***	3
MINMA	0.01172*	2								
MAXBU	-0.01338*	10								
RIBU									0.0006059*	13
RIBM	-0.0009738***	4			-0.0007603*	3			-0.0008078***	8
RIMA	0.001149*	11							0.001129***	9
RADBM	0.00000663***	1	0.00001068***	2	0.00000585*	2				
RADMA	-0.00000895*	3	-0.00002313*	1			-0.00001835**	3		
RHBU	0.015292***	8								
RHBM							0.008142*	7	0.03565***	2
RHMA	0.016115***	6							0.005912***	5
KRATIOSOIL							-9.487***	2	-1.3387*	11
R ²	76.0		74.3		48.0		85.1		99.6	
n	104		26		26		26		26	
<i>Transplanted broccoli plants</i>										
INTERCEPT	0.9355		4.526**		1.743**		1.764*		4.504**	
TXBU			-0.09219**	8						
TXMA			-1.9610**	2						
NCKB			-0.002200**	3						
PTIS									-0.1942**	7
STIS			0.00000119**	9					-0.00006016**	3
PHMA			-0.1484**	5						
MOMA									-0.001580*	9
CASOIL	0.0001223***	3	0.0002340**	1						
MGSOIL			0.00008888**	7						
MINBM	-0.03831*	1			-0.09001*	1	-0.09275*	1		
RIMA									0.002087**	4
RHBU	0.03768***	4	0.02056**	6					0.02670**	5
RHBM	-0.04444***	2	-0.03366**	4						
RHMA									-0.0672**	1
WBU									0.02343**	8
KRATIOSOIL	-0.03404*	5							-0.0179**	6
KRATIOSOIL									-6.701**	2
R ²	69.2		100.0		48.8		43.2		100.0	
n	44		11		11		11		11	

²Order of entrance of the variable in the regression model.*, **, *** Slope estimate in the final regression model is significant at $P \leq 0.1$, 0.05, 0.01, or 0.001, respectively.

in the direct-seeded plants ($r = -0.305^{**}$) and in the transplanted plants ($r = -0.542^{**}$). Within an optimum temperature range, the development rate usually increases linearly with the average air temperature (Arnold, 1959; Tan et al., 2000). Linear heat unit models have been developed for many crops under this assumption. Therefore, higher, though not extreme, air temperatures favoring the fast development of broccoli may have resulted in a decrease of brown bead incidence. Evidently, maximum temperature during the 5 d prior to maturity was not of primary importance to predict the percentage of heads with brown bead or the severity of symptoms (Tables 4 and 5).

Whereas meteorological variables were

strongly associated with brown bead incidence for transplanted broccoli plants, tissue nutrient content appeared to be more strongly associated with brown bead in the case of direct-seeded broccoli. Calcium and its antagonistic dynamics with K and Mg have already been pointed out in the few papers published on brown bead. The physiological disorder was associated with rapid growth and the resulting nutritional imbalance, including Ca deficiency (Steta, 1987). However, Pascual et al. (1996) reported that florets with brown bead had higher Ca and Mg content and lower K content, whereas the K : (Ca+Mg) ratio was higher in healthy florets. In our study, there was no evidence of a relationship between the K : (Ca+Mg) ratio and the variables character-

izing brown bead. Furthermore, low levels of Ca and high levels of K and Mg in the head tissues at maturity resulted in a high incidence and severity of brown bead in direct-seeded broccoli plants. These results confirm those of Steta (1987), who associated the incidence of brown bead with low levels of Ca in the plant and, to some extent, those of Maroto et al. (1993), who reported that foliar applications of calcium chloride decreased brown bead incidence in some cases.

Developing reproductive tissues, or vegetative tissues enclosed around older leaves, are disadvantaged in the allocation of Ca in plants (Barta and Tibbitts, 2000). Most Ca-induced disorders are caused by a general disintegration of membrane structures, with

Table 5. Stepwise multiple regressions in which the index of brown bead severity is explained by climatic and nutritional variables for seeded vs. transplanted broccoli plants, the four levels of N fertilization considered separately and all together.

Variables	All N fertilization levels		85-0-0		85-54-0		85-54-54		85-54-108	
	Estimate	Order ^z	Estimate	Order	Estimate	Order	Estimate	Order	Estimate	Order
<i>Seeded broccoli plants</i>										
INTERCEPT	-2.579*		4.713***		3.596**		4.925		-2.184	
DENS									-0.2841*	2
MGTIS	0.002288***	1			-1.876**	1	0.003142*	1	0.005109***	1
CUTIS			-0.1079*	1						
MOTIS	-2.083***	2								
PSOIL	0.01737*	4								
MGSOIL					0.00258**	2	0.00201*	4		
MNSOIL							-0.03065**	3		
RIBU							-0.01277*	5		
RADBU	0.00015***	3								
WBM							-4.335***	2		
WMA									-1.058+	3
R ²	36.8		16.0		45.8		71.4		52.9	
n	104		26		26		26		26	
<i>Transplanted broccoli plants</i>										
INTERCEPT	6.375*		26.76***		10.67**		-5.904*		18.16**	
DENS							1.202**	1		
NCKB									-0.003333**	7
NSOILB							0.01357*	2	-0.008484**	5
PTIS					10.84**	2				
CUTIS			-0.3383**	2						
FETIS					-0.00871**	4				
STIS									-0.0002343**	6
PHMA									-5.890**	4
CASOIL	0.000508*	2							0.004576**	1
MGSOIL									0.005512**	2
BSOIL			-2.604*	3	-0.05369*	8				
CUSOIL									0.6978**	3
FESOIL					-0.01689**	1				
MINBM					-0.1715**	6				
MINMA	-0.3354*	1	-0.9761**	1						
RIBM					-0.00075*	7				
RIMA					0.01080**	3				
RHBU					0.002192*	9				
RHBM									-0.02566**	8
RHMA					-0.07887**	5			0.001168*	9
R ²	27.9		86.2		100		68.1		100	
n	44		11		11		11		11	

^zOrder of entrance of the variable in the regression model.

+, **, *** Slope estimate in the final regression model is significant at $P \leq 0.1$, 0.05, 0.01, or 0.001, respectively.

meristematic tissues being affected first (Kirby and Pilbeam, 1984). Calcium is an immobile element recognized to be mainly translocated from roots through the xylem by mass flow (Clarkson, 1984). This mass flow results from transpiration, root pressure, and diurnal changes in water stress (Marschner, 1983). Most of the water flux is channeled to leaves exposed to the sun for transpiration, which contributes to the global cooling of the plant. Developing tissues fed by the phloem are often disadvantaged by this competition from transpiring tissues. Competition between sinks, such as buds, and developing leaves and fruits is high when Ca in the xylem is low and transpiration is high (Clarkson, 1984). It is then likely that broccoli florets are susceptible to Ca deficiencies. Furthermore, excessive K and Mg are likely to aggravate Ca deficiencies within the plant metabolism (Cubeta et al., 2000; Jakobsen, 1993; Mengel and Kirby, 1987).

Conditions promoting the fast development of transplanted broccoli, including high spring air temperature and a regular supply of water from the button stage to maturity, tended to decrease brown bead incidence in broccoli.

An adequate supply of N fertilization in the range of 85-54-54 or 85-54-108, along with moderate applications of K and Mg, should be considered by the grower to prevent brown bead incidence of broccoli. Whenever the development rate of broccoli is interrupted by various causes, including insufficient N, water, Ca, or unfavorable temperature conditions, brown bead will be more present in the fields. Future studies should verify whether foliar applications of Ca provide another part of the solution. Also, the relationships between flower abortion in broccoli and nutrient limitations, changes in temperature, and plant growth rates could be investigated under controlled conditions.

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