

Thiocyanate Ion Content in Relation to Clubroot Disease Severity in Cabbages¹

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Abstract. Thiocyanate ion (SCN⁻) content, derived from indolylglucosinolates, was analyzed in the head and roots of 3 cabbage lines (80-5, 80-38, and 80-35, all derived from interspecific hybridization between *Brassica napus* L. and *B. oleracea* L. Capitata group), segregating for degrees of susceptibility to clubroot disease incited by *Plasmodiophora brassicae* Wor. There was a linear increase in SCN⁻ content in heads with increasing clubroot severity of both susceptible lines 80-5 and 80-38. The head SCN⁻ content of line 80-35, resistant to clubroot, was not influenced by clubroot severity and was negatively correlated with head SCN⁻ content. Root fresh weight increased with increasing clubroot severity and was positively correlated with root SCN⁻ content.

Glucosinolates are characteristic constituents of plants of the Cruciferae. Although not harmful *per se*, glucosinolates undergo enzymic hydrolysis upon tissue disintegration yielding isothiocyanates (mustard oils), thiocyanates, and other related derivatives, known to be responsible for the characteristic flavor and pungency, as well as for the toxic and growth inhibiting properties of cruciferous plants (15). The content of thiocyanate and other glucosinolate derivatives has been related to the goitrogenic toxicity of cruciferous plants (12, 15).

The resistance of cruciferous crops to the clubroot disease incited by *Plasmodiophora brassicae* Wor. has been attributed to their content of mustard oils, an hypothesis supported by experiments indicating the antifungal properties of allylisothiocyanate and β-phenylisothiocyanate (9). However, studies (3, 4) suggested that the susceptibility to clubroot in various cruciferous crops was related to the presence of certain glucosinolates, particularly those which hydrolyse to yield thiocyanate ion (SCN⁻). Ockendon and Buczacki (11) investigated this relationship and found a significant overall correlation between the presence of glucobrassicin (3-indolylmethyl-glucosinolate) and severity of

clubroot symptom development in cruciferous crops. Mullin et al. (10) indicated that SCN⁻ content, derived from indolyl glucosinolate, did not correlate with clubroot resistance in rutabaga and turnip.

This work examines the relationship between SCN⁻ content and severity of the clubroot disease in various cabbage lines segregating with different degrees of susceptibility to this disease.

Cabbage plants of 3 segregating breeding lines 80-5, 80-38, and 80-35 were used for the chemical analysis. The lines were back-cross progenies obtained from cabbage to interspecific F₁ hybrids between rutabaga (*Brassica napus*) and 2× and 4×-cabbage (*B. oleracea* Capitata group) (5).

Seeds were sown in a cold frame on May 12, 1980, and seedlings were transplanted on June 16 in a field severely infested with the clubroot organism, *Plasmodiophora brassicae*, populations E.C.D. 16/02/30 and 16/02/31 (2) at the Agriculture Canada St-Jean

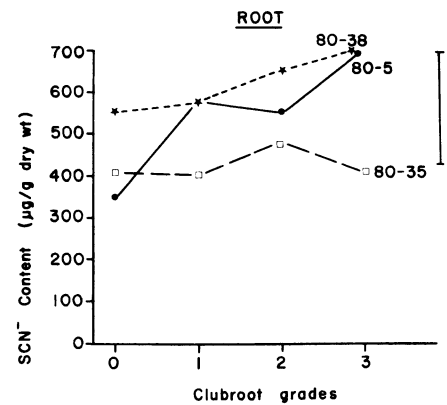


Fig. 2. SCN⁻ content in roots of 3 segregating cabbage lines in relation to clubroot severity. Vertical bars represent LSD at P = 5%.

Research Station Farm at L'Acadie, Quebec. The within-row spacing was 0.45 m in 4 replicated rows, 0.9 m apart. Plants were irrigated immediately after transplanting and cultural practices were followed according to provincial recommendations.

Whole plants of lines 80-5, 80-38 and 80-35 were harvested at the optimum marketable stage on September 3, 10, and October 2, respectively. Roots were cleaned of excess soil to facilitate examination for disease infection. Degree of root infection was rated into 4 grades according to the clubroot symptom classification of Crête (7):

Root system affected	
Grades	(%)
0	0
1	1-10
2	11-50
3	51-100

Disease index (DI, the higher the index the higher the susceptibility (8) was used as an indication of overall susceptibility. Of the 3 breeding lines, line 80-35 with DI = 48% was most resistant; line 80-38 with DI = 85% was most susceptible; and line 80-5 with DI = 65% was intermediate.

For each line, a total of 24 cabbage plants, consisting of 2 plants in triplicate samples for each of the 4 grades of clubroot symptom severity, was reserved for SCN⁻ analysis. Plants were separated into marketable head (minus the outer wrapper leaves) and root (lower infected portion). Thin cross-sectional wedges from each of the 2 heads per replicate sample were removed and combined into a 100 to 150 g sample for tissue analysis. The dry weight was determined from a parallel tissue sample. Root samples were washed, excess water was wiped off and then divided into 2 subsamples; 1 subsample was used for tissue analysis and the other for dry weight determination. Each subsample for tissue analysis was homogenized with distilled water (1 part sample: 2 parts water, w/v) in a Waring blender. The crude extract was clarified and SCN⁻ was determined colorimetrically with values expressed as µg/g KSCN per g dry weight (6). Preparation of all samples was performed on the same or the following day after the cabbages were harvested. Previous investigations indicated that aliquots from the same

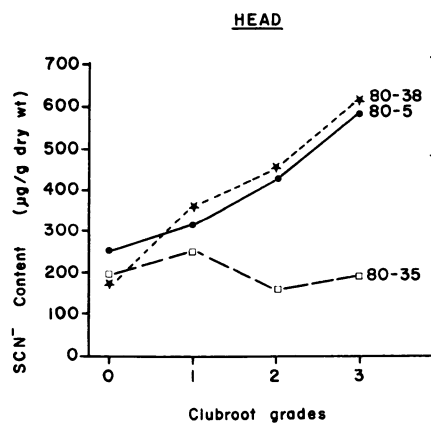


Fig. 1. SCN⁻ content in heads of 3 segregating cabbage lines in relation to clubroot severity. Vertical bars represent LSD at P = 5%.

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crude extract with pH adjusted between 3.8 and 7.4 using 15.5 N HNO₃ or 20% NaOH and incubated at different time periods up to 24 hr resulted in similar yield of SCN⁻ as corresponding non-adjusted aliquots (pH 6.2 – 6.4) (1). Data for SCN⁻ content and fresh weight of head or root were statistically analysed as a 3 × 4 × 3 factorial.

The SCN⁻ content in heads of the 3 segregating lines differed significantly (Fig. 1). There was a linear increase in SCN⁻ content with increasing clubroot severity in heads of both the 2 more susceptible lines 80-5 and 80-38, but not in heads of the least susceptible line 80-35. Similarly, the SCN⁻ content in roots of the 3 segregating lines differed significantly (Fig. 2). In roots the apparent trend towards increasing SCN⁻ content with increasing clubroot severity was not confirmed by analysis of variance.

The head fresh weight of the 3 lines showed a decreasing trend with increasing clubroot severity (Fig. 3). The reason for this decrease in head fresh weight is obvious, i.e. plant growth was disturbed by the infection of the root system. Using the combined data of the 3 lines, head fresh weight was found to be negatively correlated ($r = 0.82$, $P = 1\%$, 10 df) with head SCN⁻ content. Conversely, root fresh weight increased with increasing club-

root severity and was positively correlated ($r = 0.70$, $P = 5\%$, 10 df) with root SCN⁻ content (Fig. 4).

Glucobrassicin and neoglucobrassicin (N-methoxy-3-indolylglucosinolate) have been identified as the thiocyanate-yielding glucosinolates in cabbages (14), with glucobrassicin making the major contribution (up to 68%) of glucosinolates in this species (13). Evidence indicates that the abnormal growth symptoms of clubroot tissues are associated with higher than normal levels of auxins, which may be released from indolylglucosinolates, particularly glucobrassicin (3), but possibly also and to a lesser extent from glucotropaeolin and *p*-hydroxybenzylglucosinolate (4). The positive correlation between SCN⁻ content and fresh weight of cabbage root tissues found herein seems to offer some support of this suggestion.

Analysis of early-maturing cauliflower (*B. oleracea* L. Botrytis group cv. Imperial 10-6) revealed a significant increase in root SCN⁻ content from 271 to 636 µg/g dry weight with increasing clubroot severity (grades 0 to 3, respectively), but without affecting curds (unpublished data). This suggests that the influence of clubroot disease on glucosinolates may be manifested differently in reproductive tissues (cauliflower curds) than in vegetative tissues (heads of cabbages).

Plants of the low DI line, 80-35, had similar head SCN⁻ content among the 4 clubroot grades whereas those of lines, 80-5 and 80-38 showed increasing head SCN⁻ content with increasing severity of clubroot infection (Fig. 1).

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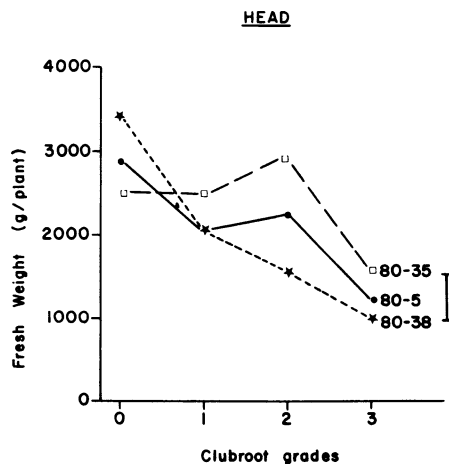


Fig. 3. Fresh weight of heads of 3 segregating cabbage lines in relation to clubroot severity. Vertical bars represent LSD at $P = 5\%$.

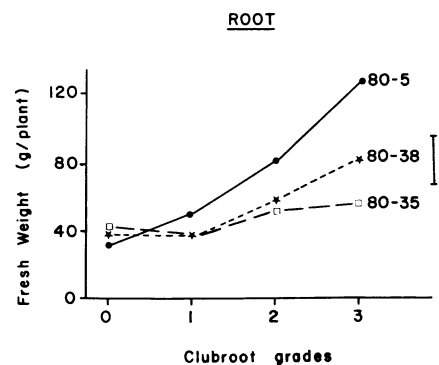


Fig. 4. Fresh weight of roots of 3 segregating cabbage lines in relation to clubroot severity. Vertical bars represent LSD at $P = 5\%$.