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Enzymatic maceration of witloof chicory by the soft rot bacteria *Erwinia carotovora* subsp. *carotovora*: the effect of nitrogen and calcium treatments of the plant on pectic enzyme production and disease development

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Abstract

Disease incidence of bacterial soft rot caused by *Erwinia carotovora* subsp. *carotovora* and activities of bacterial pectolytic enzymes were studied in witloof chicory. Disease incidence after forcing of the chicory heads was enhanced by the nitrogen treatment and reduced by the calcium treatment of the chicory plants prior to forcing. Significant differences in susceptibility to bacterial soft rot were found for the tested chicory cultivars. Disease severity after 96 h ranged from 6% in Clause R2 to 100% in Bea and Tabor. Chicory cultivars Rumba and Salsa showed a final average severity of 65–70%. Activity of the pectolytic enzymes, polygalacturonase and pectate lyase increased in artificially inoculated chicory heads of cultivar Rumba and was not affected by calcium and nitrogen treatments of the plants. Polygalacturonase showed highest activity 48 h after inoculation while pectate lyase activity increased throughout 72 h of incubation. Maceration of the chicory tissue and bacterial growth increased continuously until 96 h after inoculation, when more than 60% of the chicory heads was macerated by pectolytic enzymes of the bacteria. Enzyme activity of *Erwinia carotovora* subsp. *carotovora* grown on chicory cell wall extracts was influenced by nitrogen and calcium treatments of the chicory plants. The activity of polygalacturonase reached its highest level at 48 h after incubation and pectate lyase activity peaked at 24 h followed by a continuous decrease until 72 h after inoculation.

Introduction

Chicory is an important field-grown vegetable in the Netherlands, being the third largest chicory producer in the world with a production of 82,000 ton in 1997 (Van Kruistum, 1997). The introduction of the hydroponics system for forcing increased the occurrence of bacterial soft rot on chicory heads and losses of 50% and more of the total production occurred regularly when susceptible cultivars were used (Vantomme et al., 1989). Bacterial soft rot is characterised by maceration and cell rupture in fleshy parts of a plant. Symptoms result from disintegration of plant tissue by bacterial enzymes which are released into the tissue by soft rot bacteria (Collmer and Keen, 1986; Lei, 1985).

The extracellular pectic enzymes are the primary factors causing the rot. They initiate the maceration of plant tissue by digestion of the cell walls and the middle lamella of the cells. Subsequent cell rupture makes cellular fluids accessible for the bacteria, promoting bacterial growth which leads to increased enzymatic degradation of the tissue. The first enzymes produced by the bacteria do not require external stimuli for production or activation; they are produced constitutively (Tsuyumu and Chatterjee, 1984). Digestion products of pectate stimulate the production of other

pectic enzymes in great amounts. The enzymes then cause massive maceration of the tissue, resulting in an increase of the bacterial population and further enzyme production. The first symptoms of bacterial soft rot are browning of the tissue and visible maceration of the diseased plant parts.

Earlier studies (Knösel, 1967; Pagel, 1988) have indicated that the virulence of soft rot bacteria is closely linked to the production of pectolytic enzymes. Their production depends on the environmental conditions and the host plant involved (Zucker, 1972). Several factors affect tissue maceration, especially calcium ions and the pH of the plant tissue (Pagel and Heitefuss, 1990). Free calcium ions stimulate pectate lyase (PL) activity but inhibit polygalacturonase (PG) activity (Alghisi and Favaron, 1995).

The nitrogen content of the plants is positively correlated with the amount of nitrogen fertilisation of the chicory plants during root production (Reerink, 1993) and with the amount of bacterial soft rot (Wright, 1993). This study focuses on the effects of the nitrogen content in chicory roots and the application of calcium chloride as a root immersion treatment on the amount of maceration caused by soft rot bacteria. Nitrogen and calcium contents of the crop are varied by standard cultural practices. The effects of nitrogen and calcium status of the crop on the enzyme activity of the bacteria are studied as well as the consequences on soft rot development during forcing of the chicory heads of several cultivars. Moreover, we compare the activity of the pectolytic enzymes in inoculated chicory heads and in cell wall extracts from chicory heads as a pectate source.

Material and methods

Plant material

The crop was produced following standard practices at the Experimental Station for Arable Farming and Vegetables as described previously (Schober and Zadoks, 1998). Witloof chicory cultivars Salsa, Rumba, Tabor, Bea and Clause R2 were used in the experiments. The soil of the experimental fields was a loamy sea-clay with 1.8% organic matter and 5.8% calcium (all measured in the dry matter). Nitrogen fertilisation (indicated as high nitrogen) was applied as NH_4NO_3 during the root production stage in two applications, one as a pre-sowing application of 70 kg ha^{-1} and an additional top dressing in June to a total of 140 kg ha^{-1} mineral nitrogen in the topsoil (0–0.30 m). In August, mineral nitrogen in the topsoil was supplemented to a total of 200 kg ha^{-1} nitrogen with an additional top dressing. The calcium treatment (indicated as high calcium) was applied as a root immersion treatment in a 3% aqueous solution of calcium chloride directly after harvest of the roots in late September.

Roots were harvested into bins of approximately 1.5 m^3 and were immersed in the calcium chloride solution in these bins for 3 min. Chicory heads were produced at three forcing seasons, early in November, intermediate in December and late in March using temperature-controlled forcing cells, forcing lasted for 20–23 days. To produce the heads, chicory roots were packed in wooden trays with each tray containing 300–350 roots from one combination of the nitrogen and calcium chloride treatments. Water and air temperatures of the forcing cells varied with the forcing season according to standard practice to optimise head growth and quality (Van Kruistum, 1997). The trays containing the roots from the four nitrogen and calcium treatment combinations were placed in four stacks and each stack was connected to a recirculation unit of nutrient solution. Chicory heads of cultivars Bea, ClauseR2, Salsa and Tabor were produced under the low nitrogen and low calcium treatment for the intermediate and late forcing season, whereas cultivar Rumba was produced under both nitrogen and calcium treatments for all forcing seasons.

Cell wall extracts from freshly harvested chicory heads of the intermediate and late forcing season of cultivar Rumba were prepared according to McGuire and Kelman (1986) and Voragen et al. (1980) by acid alcohol extraction of the cell walls from chicory pulp. Calcium contents of freshly harvested chicory heads and of cell wall extracts of the heads from cultivar Rumba from the early and intermediate forcing seasons were measured by the Chemical Laboratory of the Research Institute for Agrobiology and Soil Fertility (AB-DLO) using a flame spectrophotometer.

Bacteria

Erwinia carotovora subsp. *carotovora* (Jones) Bergey et al. 1923 (*Ecc*) strain nr. PD-1053 from witloof chicory cultivar 'Flash' was used in these experiments. Cultures were grown at 27 °C both in nutrient broth (NB) and nutrient agar (NA). Cells were directly harvested from broth or suspended in sterile water (Milli Q) from agar plates using 2–3 day old cultures.

Suspensions were centrifuged for 10 min at 12,000 rpm (Sigma centrifuge) and the pellet was suspended to obtain appropriate densities for inoculations. Densities were assessed by turbidity measurement using a colorimeter at 600 nm.

Inoculation of chicory heads and cell wall extracts

The outer leaves of harvested witloof chicory heads were removed aseptically and the heads were weighed. Chicory heads were surface sterilised with 70% ethanol and one leaf was removed with sterile equipment, causing a wound for artificial inoculation. The bacterial suspension (15 µl of a suspension with $1.5 * 10^5$ cfu ml^{-1}) was applied to the wound. Inoculated chicory heads were placed in sterile plastic boxes, boxes were closed with a lid and incubated at 25 °C. Plant cell wall extracts of cultivar Rumba were suspended in protein extract medium (PEM) prepared according to Klement (1990) with the omission of all carbon sources (2 g cell wall extract in 25 ml PEM). Media were prepared separately for both calcium and nitrogen treatments of the cell wall extracts and inoculated with *Ecc* to a final density of $5 * 10^6$ cfu ml^{-1}.

Assessment of disease

The amount of naturally occurring soft rot was assessed directly on the chicory heads of cultivar Rumba for early, intermediate and late forcing from 1994 until 1997 to a total of 12 assessed forcing seasons. Disease incidence (fraction of diseased heads) was used for statistical analysis.

The amount of maceration in artificially inoculated chicory heads was measured daily until 4 days after inoculation. Rotting tissue was removed from the chicory heads with a sterile spatula. The macerated tissue and the remaining healthy tissue were weighed to quantify disease severity. 2 g of macerated tissue were suspended in sterile water and the turbidity of the suspension measured at 600 nm. Samples of 15 µl were serially diluted and plated on tryptic soy agar (TSA). Plates were incubated at 27 °C until colonies were clearly visible. Bacterial density was assessed daily and expressed as the number of cfu's per gram of macerated tissue.

Measurement of enzyme activity

Activity of pectolytic enzymes of *Ecc* was measured both in plant tissue and cell wall extracts from chicory heads from cultivar Rumba. The effects of the nitrogen and calcium treatments of the chicory roots on the activity of the enzymes were compared using plant tissue and cell wall extracts from freshly harvested chicory heads.

Measurement of enzyme activity in chicory heads inoculated with Ecc

After removal of the brown and rotting chicory tissue, a 1 g sample from the margin of a lesion was cut from the chicory head. Samples were weighed, cut into small pieces and put in 2 ml sterile water (Milli Q). Samples were gently agitated to release intercellular fluids. For direct assessments the extracts were filter-sterilised with a 0.2 µm filter (Redrim, Schleicher and Schuell). Samples of the suspensions were used for acetone precipitation as described below.

Measurement of enzyme activity in artificial medium with cell wall extracts inoculated with Ecc

Samples of 3 ml from the liquid cultures were centrifuged at 12,000 rpm to remove bacterial cells and used directly for acetone precipitation. For acetone precipitation, the samples were precipitated with 4 volumes of acetone at −20 °C for 1 h, centrifuged at 12,000 rpm for 10 min (Sigma centrifuge) and the pellet was washed twice in 80% acetone. Measurements were performed at the optimum pH of the two enzymes (pH 5 for PG and at pH 8 for PL). The pellet was suspended in the appropriate buffer (0.1 M sodium acetate, pH 5 for PG or 50 mM Tris-HCl, pH 8 for PL). Presence of enzymes was verified at 235 nm in a spectrophotometer according to Knösel and Lange (1971). PG activity was assessed using the modified Nelson's assay (Easton and Rosall, 1985). PL activity was measured at 235 nm in a spectrophotometer (Collmer et al., 1988). Enzyme activity was measured at 12, 24, 48 and 72 h after inoculation in two replications per sampling time. One unit of activity of the enzymes was defined as the amount of enzyme liberating 1 µmol of reducing groups min-1 at 25 °C.

Statistical analysis

Disease incidence after forcing of witloof chicory Rumba was analysed after arcsine$\sqrt{}$-transformation of the data. Data from plots with visible symptoms of disease in the chicory heads (incidence > 0) were used

for analysis after being pooled over the four years of the experiment. Tissue maceration, growth and enzyme activity of *Ecc* were measured daily until 4 days after inoculation for the artificially inoculated chicory heads of cultivar Rumba. All assessments were performed in two replications per nitrogen and calcium treatment and per sampling time ($n = 8$). Bacterial densities were log-transformed. Enzyme activity of *Ecc* in artificial media with cell wall extracts from cultivar Rumba as a pectate source were measured daily until 4 days (72 h) after inoculation.

Results

Disease incidence after forcing of the heads

Bacterial soft rot occurred during all three forcing seasons in the years 1994–1997, no significant differences were found for the disease incidence between the years (Table 1). The average disease incidence ranged from 35% in the early forcing season to 31% in the intermediate and late forcing season. Application of the calcium treatment reduced the average disease incidence to 8% in the early, 31% in the intermediate and 23% in the late forcing seasons. The nitrogen treatment of the roots increased the nitrogen content in the dry matter of the roots, roots from the high nitrogen treatment contained an average of 10.1 g kg^{-1} dry matter and roots from the low nitrogen treatment an average of 4.7 g kg^{-1}. The disease incidence in the high nitrogen treatment was 6% higher in the early forcing and 13% higher in the intermediate and late forcing season than incidence in the low nitrogen treatment. Highest disease incidence was found for the low calcium–high nitrogen combination for all three forcing seasons.

The treatment of low calcium–low nitrogen combination resulted in a significantly higher disease incidence during the intermediate and the late forcing seasons than during the early forcing season. The treatment of calcium–nitrogen and calcium-forcing season combinations were significant (Table 2). Disease incidence in chicory heads changed not only with the nitrogen fertilisation of the roots but also during the forcing seasons from early to intermediate and late forcing. Both nitrogen and calcium treatments had a clear effect on the nitrogen and calcium content of the harvested chicory heads (Table 3). Heads from the high nitrogen– and high calcium treatment combination had the highest calcium and phosphate content, followed by the low nitrogen and high calcium treatment combination. Heads from the low nitrogen content had the lowest calcium content. Potassium was high in the low nitrogen treatments and was not affected by the calcium treatments.

Table 2. Significance levels from ANOVA for the soft rot incidence after forcing of chicory heads from cultivar Rumba for three forcing seasons during 1994–1997 for two calcium and nitrogen treatments of the roots prior to forcing applied as described in Material and methods (incidence > 0, arcsine$\sqrt{}$-transformed)

Source	*df*	Significance levels
Calcium	1	<0.000
Nitrogen	1	<0.000
Forcing season	2	0.005
Calcium–Nitrogen	1	0.007
Calcium-Forcing season	2	<0.000
Nitrogen-Forcing season	2	0.320
Error	759	

Table 1. Average disease incidence (fraction diseased heads) and standard errors after forcing from cultivar Rumba for three forcing seasons of witloof chicory with two calcium and two nitrogen treatments of the roots prior to forcing applied as described in Material and methods (incidence > 0, arcsine$\sqrt{}$-transformed)

Calcium treatment	Nitrogen treatment	Disease incidence		
		Forcing season		
		Early	Intermediate	Late
High	High	0.33 ± 0.15 a[1]	0.33 ± 0.13 a	0.32 ± 0.10 a
	Low	0.36 ± 0.13 a	0.29 ± 0.10 a	0.30 ± 0.11 a
Low	High	0.40 ± 0.17 a	0.52 ± 0.20 b	0.44 ± 0.16 b
	Low	0.34 ± 0.15 a	0.45 ± 0.19 b	0.36 ± 0.15 a

[1]Values in a column not followed by a common letter are significantly different at $p \leq 0.05$ according to Tukey's HSD test.

Table 3. Mineral analysis of chicory heads cultivar Rumba from the forcing in 1998 from two nitrogen and two calcium treatments of the roots prior to forcing applied as described in Material and Methods. Data are average content and its standard error ($N = 32$)

Treatments		Mineral analysis of chicory heads in g kg^{-1} dry weight			
Calcium treatment	Nitrogen treatment	Calcium	Nitrogen	Phosphate	Potassium
Low	Low	2.3 ± 0.16 a[1]	5.5 ± 0.49 a	2.4 ± 0.19 ab	26.4 ± 1.22 a
High	Low	2.5 ± 0.15 ab	n.d.	2.2 ± 0.13 b	24.5 ± 1.79 a
Low	High	2.7 ± 0.41 b	10.9 ± 0.42 b	2.4 ± 0.11 ab	23.1 ± 0.56 b
High	High	3.2 ± 0.46 c	n.d.	2.5 ± 0.18 a	24.8 ± 2.18 b

[1]Values in a column not followed by a common letter are significantly different at $p \leq 0.05$ according to Tukey's HSD test.

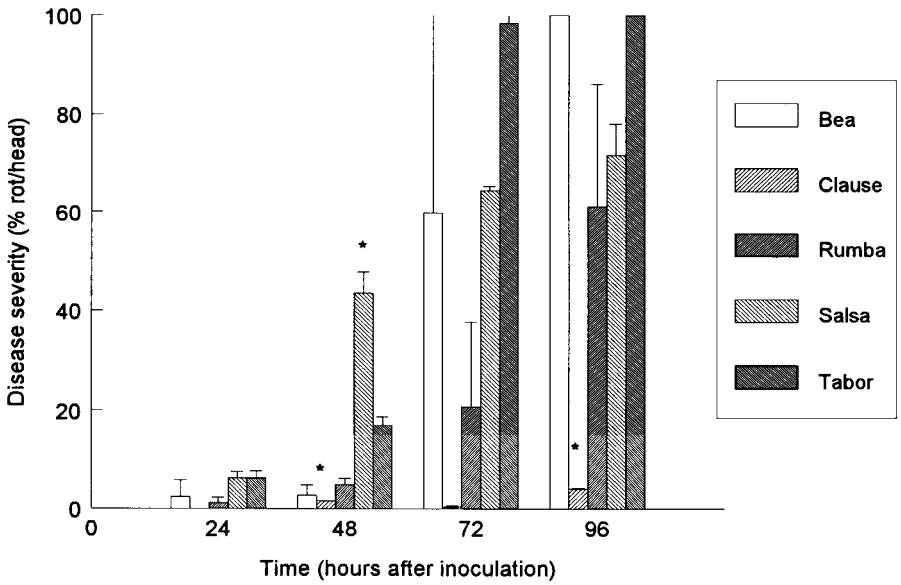

Figure 1. Disease severity (fraction of diseased heads) after inoculation of several cultivars with *Ecc* (15 µl, $1.5 * 10^5$ cfu ml^{-1}). Chicory heads were incubated at 27°C for 4 days and severity assessed daily. Asterisks (*) indicate a significant difference at that time and bars represent the standard deviation of the mean.

Disease severity in several chicory cultivars

Chicory heads from several cultivars and forced from roots of the low nitrogen and calcium treatment showed a different disease severity for the cultivars (Figure 1). Disease severity increased exponentially for all cultivars during the incubation period. The disease progressed most rapidly in Salsa (significantly highest severity after 48 h) and slowest in Clause (significantly lowest severity after 96 h). After the final observation at 96 h, cultivars Tabor and Bea were macerated almost completely, cultivars Rumba and Salsa were macerated for more than 60% and cultivar Clause R2 only for 6%. The progress of the disease in the chicory heads was different for the cultivars and the final observation of disease severity, 96 h after inoculation, was significantly different for the chicory Clause R2 compared to the other cultivars. After the incubation period, disease severity was more than 50% in all cultivars except for Clause R2.

Disease severity and enzyme activity of Ecc *inoculated on chicory heads of cultivar Rumba*

Disease severity increased exponentially and coincided with an exponential growth pattern of the bacteria in the macerated tissue of the chicory heads (Figure 2). Tissue maceration of the chicory heads increased during the incubation period up to an average severity of 60% after 96 h when the bacterial population reached its stationary phase at a density of $5 * 10^9$ cfu g^{-1} macerated tissue. PL activity was detected early (12 h after inoculation (hpi)) and levels did not increase substantially until 48 hpi (Figure 2). After this time a second increase in PL activity was observed. PG activity was first detected only at 24 hpi and increased to a maximum level of 48 hpi. Between 48 and 72 hpi PG activity decreased by 50% in spite of a fivefold increase in bacterial density in the macerated tissue.

Enzyme activity of Ecc *cultures in artificial media with cell wall extracts of cultivar Rumba*

The calcium content of the cell wall extracts was measured for the four treatment combinations. The calcium content of the cell wall extracts was significantly different for the two calcium treatments but was not influenced significantly by the nitrogen treatment. The calcium content of the cell wall extracts was on average 8.5 g kg^{-1} and varied from 5.9 g calcium kg^{-1} in cell wall extract from the high nitrogen and low calcium treatment combination to 10.9 g kg^{-1} in cell wall extract from the low nitrogen and high calcium treatment combination.

Ecc cultures in artificial media with cell wall extracts increased exponentially during the incubation period (Figure 3). Bacterial densities increased from $5 * 10^6$ cfu ml^{-1} at time = 0 h to 10^{10} cfu ml^{-1} in the stationary state 24–48 h after inoculation. The growth of *Ecc* grown in media with cell wall extracts was not significantly affected by the two nitrogen and calcium treatments of plants from which the cell wall extracts were made. The highest activity of PL coincided with the end of the exponential growth stage of the bacteria after 24 h and enzyme activity subsequently dropped.

The transient course of the PL activity in cell wall extracts was found in artificial media with various commercially available pectin sources (data not shown). The activity of PL was higher using cell wall extract from the high nitrogen treatment than from the low

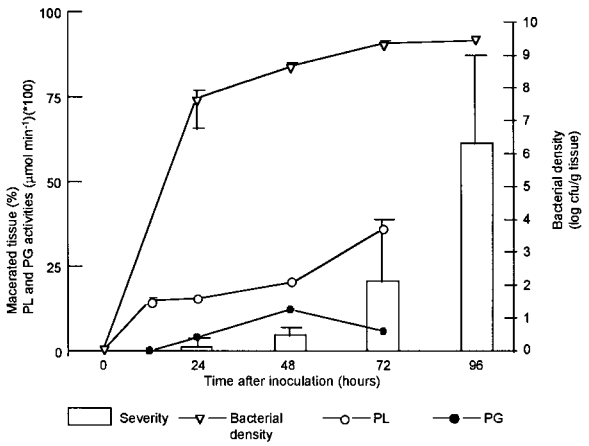

Figure 2. Growth of *Ecc* (right *y*-axis) and percentage of macerated tissue (left *y*-axis) of witloof chicory heads of cultivar Rumba from the low nitrogen and calcium treatment after artificial inoculation with *Ecc* (15 µl, $1.5 * 10^5$ cfu ml^{-1}). Activity of the pectolytic enzymes PL and PG (*100) is indicated on the left *y*-axis.

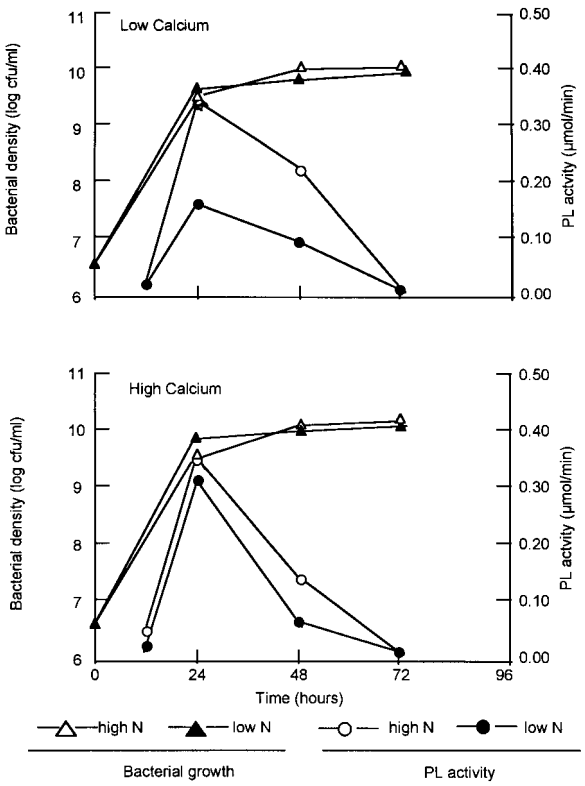

Figure 3. Growth of *Ecc* on cell wall extracts from chicory cultivar Rumba in PEM medium (left *y*-axis) and activity of the pectolytic enzyme PL (right *y*-axis) from two calcium and nitrogen treatments of the chicory heads prior to cell wall extraction as described in Material and methods.

nitrogen treatment but decreased in both cultures after reaching the stationary phase. PG activity reached the highest activity 48 h after inoculation. The activity of both PL and PG of *Ecc* grown in cell wall extracts from the low nitrogen treatment differed significantly for the two calcium treatments at 24 h after inoculation.

Discussion

Ecc is a soft rot pathogen and its virulence is related to the amount of macerated tissue caused by the production of extracellular pectolytic enzymes (Pérombelon and Salmond, 1995). During the infection process, several steps occur before soft rot symptoms appear. The bacteria present in the intercellular spaces of the chicory tissue produce constitutive pectolytic enzymes. These exo-enzymes cleave structural pectic polymers in the plant cell wall and middle lamella of the plant, facilitating the penetration and colonisation of the tissue (Collmer and Keen, 1986). Products of the pectate digestion activate a regulatory mechanism in the bacteria which stimulates synthesis of additional extracellular pectolytic iso-enzymes (Alghisi and Favaron, 1995). We hypothesised that the influences of cultural practices on disease incidence and severity in the crop is mediated by influencing the enzymes involved in pathogenesis. Free calcium in the form of bivalent ions is necessary for the activation of PL but inhibits PG (Pagel and Heitefuss, 1990). Calcium is predominantly incorporated in pectic polymers of the plant cell walls and middle lamellae (Barras et al., 1994) to form an 'egg-box' structure with pectic substances. This structure is almost inaccessible for pectolytic enzymes (McGuire and Kelman, 1986) which results in a reduced cleavage by the bacterial enzymes.

To test the influence of cultural practices on the enzymatic degradation of the plant pectate, we used chicory grown under different nitrogen and calcium treatments as growth media for *Ecc*. The transient course of the PL activity of *Ecc* in liquid culture containing cell wall extracts from our experiments was different from the continuous increase of the PL activity in artificially inoculated chicory heads (Figures 2 and 3). The continuous increase of PL activity in plant tissue may be caused by the transcription of several gene families of PL, which are regulated differently *in vitro* and *in planta*. Yang et al. (1992) have shown that accumulation of mRNAs for PL in *Ecc* was sequential and correlated with rotting of the plant material and did not occur in liquid culture of *Ecc* using polygalacturonic acid as a carbon source. In cultures of *Ecc* grown on cell wall extracts, the temporal pattern of PL activity but not of the PG activity differed from the enzyme activity as measured in *Ecc* grown on chicory heads.

The growth media containing only cell wall extracts provide only a polymeric carbon-source for the soft rot bacteria, whereas in macerating tissue the cell walls rupture, providing easily digestible nutrients for the bacteria in addition to the cell wall fragments. The induction of pectolytic enzymes is known to vary between studies *in vitro* and *in planta* (Bateman and Millar, 1966; Alghisi and Favaron, 1995). The lack of metabolites of the pectate degradation in artificial media, especially of 2-keto-3-deoxygluconate (KDG), is directly interfering with the repression of genes encoding for PL iso-enzymes (Pérombelon and Salmond, 1995). PG is inhibited by plant extracts of canola and the extent of inhibition is directly related to the calcium contents of the plant extracts (Annis and Goodwin, 1997).

The forcing season, nitrogen and calcium treatment had a significant effect on disease incidence of chicory heads. During the intermediate and late forcing seasons, more disease were found in chicory heads from the high nitrogen and low calcium treatments. Using artificially inoculated chicory heads to measure the activity of the pectolytic enzymes PG and PL, we found no differences in the enzyme activity between the nitrogen and calcium treatments. Nitrogen levels in the crop are known to influence soft rot severity (Bartz et al., 1979; Canaday and Wyatt, 1992; Carballo et al., 1994). Nitrogen fertilisation increases the dry matter content of broccoli (Everaarts, 1994) and has a direct effect on the cell wall composition, especially the esterification of pectate in the cell walls. In addition, nitrogen fertilisation interacts with the production of plant defence substances as phenols and it inhibits calcium uptake (Reerink, 1993).

Different chicory cultivars showed different susceptibility to soft rot. At the end of the incubation period, severity varied from 100% macerated tissue in Tabor to 6% in Clause R2. The high variation between replications during this experiments is a point of concern. Even in large experiments performed under highly conditioned circumstances the variance of the results are such that only weakly significant results appear. Part of the variation can be explained by the time delayed effects of the applied treatments. Nitrogen fertilisation is applied during the root production, 2–8 months before forcing. Calcium immersion of the roots is applied directly after harvest of the roots in September. The effects both of

nitrogen and calcium on the soft rot incidence and the disease severity after forcing of the chicory heads are most probably connected with other factors, especially the physiological condition of the chicory roots during forcing. More research is needed to link effects of cultural practices to differences in cell wall composition. Pérombelon and Salmond (1995) found a correlation between the resistance of potatoes against bacterial soft rot and the composition of the plant cell walls. Soft rot severity caused by *Ecc* could be reduced when either the bacterial multiplication rate or the activity of the pectolytic enzymes was reduced. The growth rate is directly linked to the availability of nutrients, including those from enzymatic breakdown of the cell walls. Cultural measures are known which can reduce bacterial soft rot in spite of the presence of the pathogen. Such measures are most likely to interact on the level of enzymatic breakdown of the cell walls. Analogous to potato cultivars, the resistance of witloof chicory cultivars against bacterial soft rot may be linked to the ability of the cultivars to incorporate calcium in the cell walls. Potato cultivars resistant to bacterial soft rot had consistently higher amounts of calcium in cell wall preparations than susceptible cultivars and they had increased levels of galacturonic acid in cell walls (McGuire and Kelman, 1986). An increasing calcium fertilisation of the tubers increased the deposits of calcium in the cell walls and improved the stability of the cell. They concluded that as calcium inhibits the primary pathogenicity factor of soft rot bacteria, the pectolytic enzymes, calcium uptake may be at least partially responsible for resistance against bacterial soft rot.

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