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# Charakterisierung und Modellierung des Abreifeverhaltens von Silomaisgenotypen mittels futterwertbestimmender Parameter

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# Kapitel 1

# Einleitung

Die Bedeutung von Silomais (*Zea mays* L.) in der Wiederkäuerfütterung hat in den letzten Jahrzehnten durch die züchterische Anpassung an kühlere Klimate in Nord-West Europa stetig zugenommen. Das hohe Leistungspotential der Pflanzen, die Mechanisierbarkeit der Produktion und die meist problemlose Silierbarkeit untermauern die Vorzüglichkeit von Silomais im Vergleich zu anderen Futterpflanzen. Seit dem Inkrafttreten des Erneuerbare-Energie-Gesetzes (EEG) im Jahre 2000 und besonders nach der Novellierung des Gesetzes im Jahre 2004 (Anonymus, 2004), wird Mais auch in verstärktem Maße zur Wärme- und Energiegewinnung, z.B. durch die Fermentation in Biogasanlagen genutzt. Im Jahre 2005 wurde auf einer Fläche von ca. 1.26 Mio. Hektar (ha) Silomais angebaut, die Anbaufläche von Mais zur Strom- und Wärmegewinnung ist von rund 10 500 ha im Jahr 2004 auf voraussichtlich 140 000 ha im Jahr 2006 gestiegen (Anonymus, 2006a).

Die Tatsache, dass in der Europäischen Union zur Zeit etwa 1100 Maissorten im Gemeinsamen Sortenkatalog eingetragen sind (Anonymus, 2006b) und in Deutschland für etwa 350 Maissorten (ca. 220 Silomaissorten) Sortenzulassungen gelten (Anonymus, 2006c), zeigt das große züchterische Interesse an der Pflanze Mais.

Die Klassifizierung von Silomaissorten erfolgt seit 1998 entsprechend ihrer Nutzungsrichtung (Siloreife- bzw. Kornreifezahl; Mitner und Rath, 1998) und orientiert sich für Silomais an dem Trockensubstanz (TS)-Gehalt der Gesamtpflanze. In Landessortenversuchen erfolgt weiterhin eine Beurteilung von Silomaissorten anhand des relativen Trockenmasse (TM)- Ertrages, bzw. des TS-, Stärke- und Energie-Gehaltes. Weitere Qualitätsparameter werden dagegen nicht zur Sortenempfehlung herangezogen. Eine genaue Charakterisierung von Silomaissorten anhand von Qualitätsparametern würde den Züchtern die Möglichkeit eröffnen, die genetische Variabilität stärker zu nutzen und in Form neuer Sorten in entsprechende Marktnischen zu positionieren. Ferner würde sich für die Landwirte die Gelegenheit bieten, die Sortenwahl auf spezifische Anforderungen abstimmen zu können.

*Futterwertbestimmung von Silomais – methodische Aspekte*

Maissilage stellt ein qualitativ hochwertiges Grundfuttermittel dar und kann in Kombination mit Grassilage dem Energie- und Proteinbedarf von hochleistenden Wiederkäuern entsprechen. Bei begrenzter Trockenmasse-Aufnahme sollte daher, neben dem Flächen- oder Energie-Ertrag, der Qualität von Silomais höchste Priorität zuteil werden. Minderqualität

muss durch erhöhte Kraftfutterzugaben kompensiert werden, welche zu ökonomischen Einbußen führen können (Kelm, 2004).

Aus ernährungsphysiologischer Sicht bietet Maisstärke gegenüber anderen Getreidestärken (z.B. Gerste, Weizen) in der Wiederkäuerernährung den Vorteil einer langsameren Fermentation im Pansen (Waldo, 1973; Nocek und Tamminga, 1991; Huntigton, 1997). Dies führt zu einer erhöhten Anflutung von Glucose am Dünndarm, wo die energetische Verwertung der Stärke effizienter erfolgt als im Pansen (Owens et al., 1986). Die Höhe des Stärkeabbaus im gesamten Verdauungstrakt (bis zu 100%; Jensen et al., 2005) ist in erheblichem Maße abhängig von der Vorbehandlung, vom Abreifegrad und der Textur des Korns (Phillippeau et al., 1999; Correa et al., 2002; Verbic et al., 2005).
Neben der Stärke determinieren sowohl die Gehalte als auch die Zusammensetzung der Zellwandbestandteile (NDF (neutral detergent fiber), ADF (acid detergent fiber), Hemicellulose, Cellulose und Lignin) und ihre Verdaulichkeiten den Futterwert der Mais-Gesamtpflanze (Jung und Deetz, 1993, Jensen et al., 2005). Auch dem Abreifegrad der Pflanzen kommt dabei eine große Bedeutung zu (Cone und Engels, 1993; Jung et al., 1998; Di Marco et al., 2002; Jung und Casler, 2006 a, b).

Zur Futterrationsbestimmung für Wiederkäuer ist die genaue Quantifizierung und analytische Charakterisierung vor allem der Kohlenhydratfraktionen in Futtermitteln und dabei insbesondere eine genauere Erfassung aller Zellwandbestandteile erforderlich (Südekum, 2001). Die Bestimmung des ernährungsphysiologischen Wertes von Futtermitteln wird oftmals mittels Weender Futtermittelanalyse durchgeführt (GfE, 2001). Diese erfasst Stoffgruppen, die in der chemischen Zusammensetzung heterogen sind und dem physiologischen Wert für Wiederkäuer nicht entsprechen (Kirchgeßner, 1987). So werden beispielsweise die Zellwandbestandteile sowohl in die Fraktion 'Rohfaser' als auch in 'N-freie Extraktstoffe' eingeteilt.
Ferner ist der Hohenheimer-Futterwerttest nach Menke und Steingass (1988) eine weit verbreitete Analyse zur Schätzung des energetischen Futterwertes. Vornehmlich Kohlenhydratfraktionen werden in unterschiedlichen Raten bei der Inkubation mit Pansensaft von Pansenmikroben abgebaut (Cone et al., 1997). Die gebildete Gasmenge steht im engen Zusammenhang zu in vivo gemessenen Parametern (Blümmel und Ørskov, 1993). Trotz

Effizienzsteigerungen gegenüber in situ Methoden (Mehrez und Ørskov, 1977) verhindert der immer noch relativ hohe Zeit- und Kostenaufwand, bzw. der Bedarf fistulierter Wiederkäuer den routinemäßigen Einsatz dieser Methode.

Eine schnelle und kostengünstige Alternative zur naßchemischen Bestimmung der Futterqualität stellt die Nah-Infrarot-Reflexions-Spektroskopie (NIRS) dar. Diese Methode beruht auf der frequenzspezifischen Absorption von molekularen Strukturen, v. a. OH-, NH-, CH- und CO-Bindungen, sodass Absorptionswerte in ihrer Gesamtheit zur quantitativen Analyse von chemischen Inhaltsstoffen herangezogen werden können (Mainka, 1990). Anorganische Stoffe (Mineralstoffe, Spurenelemente, Asche) können mittels NIRS nur unzureichend genau bestimmt werden (Clark et al., 1989), jedoch ist die NIRS-Methode zur Bestimmung von organischen Inhaltsstoffen (Protein, Stärke, Fett, usw.) in der Praxis etabliert (Murray, 1993; Givens et al., 1997; Stuth et al., 2003).

Während die Anwendbarkeit der NIRS auch bei biologischen Parametern durch in vitro, in situ und in vivo Studien belegt wurde (Givens et al., 1997; Stuth et al., 2003), lassen Untersuchungen zur Schätzung der Gasbildung von Silomais Unzulänglichkeiten erkennen (Lovett et al., 2004). Mit geringen Bestimmtheitsmaßen und hohen Schätzfehlern ist momentan eine gesicherte Aussage über die Gasbildungskinetik mittels NIRS nicht möglich, welches auf die Heterogenität der Maispflanze zurückgeführt wird (Lovett et al., 2004).

*Einfluss der phänologischen Entwicklung auf Futterqualitätsmerkmale*

Die Entwicklung von Silomais ist durch Veränderungen der Inhaltsstoffe, besonders der Kohlenhydratfraktionen charakterisiert, welche der Verschiebung der morphologischen Zusammensetzung folgt (McAllen und Phipps, 1977; Phipps und Weller, 1979).

In der vegetativen Entwicklung wird zunächst der Blattapparat der Maispflanzen ausgebildet und im Zuge der stattfindenden Photosynthese werden Assimilate, bestehend aus wasserlöslichen Kohlenhydraten (WLK), vornehmlich Fructose und Glucose, produziert (Stewart et al., 2003). Diese werden im Speichergewebe von Blättern und v. a. im Stängel zwischengelagert (Phipps et al., 1984; Uhart und Andrade, 1995) und nach der weiblichen Blüte in Form von Saccharose zu den sich entwickelnden Kolben transloziert (Hawker et al., 1991; Kühn et al., 1999), in denen die Stärkebildung (Martin und Smith, 1985) stattfindet. In Folge der Stärkeakkumulation erfolgt eine Zunahme des TS-Gehaltes im Kolben, der sich in steigenden

TS-Gehalten der Gesamtpflanze widerspiegelt (Ma et al., 2006). Bei fortschreitender Abreife sinken die absoluten Gehalte an WLK in der Rest- und Gesamtpflanze (Russell, 1986; Oldenburg und Laws, 1993), während die Gehalte an Gerüstsubstanzen, wie NDF, ADF, Hemicellulose, Cellulose und Lignin, in der Restpflanze zunehmen (Irlbeck et al., 1993; Verbic et al., 1995; Tolera und Sunstol, 1999). In der Gesamtpflanze werden die zunehmenden Gehalte an Zellwandbestandteilen durch den steigenden Kolbenanteil und somit durch den steigenden Stärkegehalt überlagert und sinken bis zur Siloreife (Bal et al., 1997; Di Marco, 2002). Im späteren Verlauf der Abreife ist keine weitere Abnahme im Gerüstsubstanzgehalt festzustellen (Wiersma et al., 1993; Bal et al., 1997), da sich die beiden Effekte gegenseitig aufheben.

*Einflussfaktoren auf die Qualitätsentwicklung - genetische Variabilität*

Nach Einführung der nutzungsspezifischen Klassifizierung von Maissorten wird die Eignung von Silomaissorten zur Produktion qualitativ hochwertiger Silage nicht nur mit dem Kolbenanteil gleichgesetzt, sondern auch der Bedeutung der Restpflanze Rechnung getragen. Daher fokussieren die Pflanzenzüchter ihre Arbeit in den letzten Jahren verstärkt auf den Futterwert der vegetativen Pflanzenbestandteile.

Zur Erhöhung des Futterwertes der Restpflanze und somit der Gesamtpflanze wurden neuartige Sortentypen entwickelt. So genannte bm3, 'leafy' und 'lax leaf' Hybriden zeigen höhere Verdaulichkeiten, da sie einen reduzierten Ligningehalt bzw. einen höheren Anteil an Blättern, die im Vergleich zu dem Stängel besser verdaulich sind, aufweisen (Tine et al., 2001; Falkner et al., 2000; Andrews et al., 2000). Risiken bezüglich der Standfestigkeit und hohe Saatgutkosten, bei gleichzeitig niedrigerem Flächenertrag und derzeit widersprüchlichen Auswirkungen auf die tierische Leistung verhindern bisher jedoch die Eingliederung in den deutschen/europäischen Saatgutmarkt (Tjardes et al., 2000; Cox und Cherney, 2001; Barrière et al., 2003, Pedersen et al., 2005).

Etablierte Sorten weisen häufig eine asynchrone Abreife der Restpflanze im Vergleich zum Kolben auf, jedoch zeigen auch sogenannte 'stay green' Sorten (Thomas und Howarth, 2000), die eine verzögerte Restpflanzenabreife aufweisen, keine Vorteile bezüglich ihrer Verdaulichkeit (Ettle und Schwarz, 2003). Allerdings werden höhere Erträge dieser Sorten durch gesteigerte Photosyntheseleistungen beschrieben (Valentinuz und Tollenaar, 2004).

Bei einem Vergleich von 'neueren' (Sortenspektrum der 1990er Jahre) im Vergleich zu 'alten' (Sortenspektrum der 1970-80er Jahre) Silomaissorten werden aufgrund der früheren Fokussierung auf den Kolben abnehmende Zellwandverdaulichkeiten von Silomaissorten beschrieben (Givens und Deaville, 2001). Dagegen werden eine bessere Anpassung an marginale Anbaugebiete v. a. durch höhere Stresstoleranz (Tollenaar und Wu, 1999) und frühere Reife ebenso wie höhere Stärkegehalte (Frei, 2000; Givens und Deaville, 2001) als Züchtungserfolge bewertet.

Vielfach wurden konventionelle Sorten auf Unterschiede bezüglich der Qualitätsparameter und tierischen Leistung untersucht, jedoch führten diese Untersuchungen zu gegensätzlichen Aussagen. Sortenunterschiede in Qualitätsparametern, v. a. in Gerüstsubstanzgehalten (u. a. Allen et al., 1991; Hunt et al., 1993; Cox et al., 1994) bzw. im Futterwert sind dokumentiert (u. a. Barrière et al., 1997; Argillier et al., 2000). Andere Studien konnten nur geringfügige oder keine Unterschiede in den Gehalten von Inhaltsstoffen nachweisen (u. a. Andrieu et al., 1993; Irlbeck et al., 1993; Darby und Lauer, 2002).
Das asynchrone Abreifen von Kolben und Restpflanze neuerer Sorten kann zum einen der Bestimmung des Erntezeitpunktes entgegenstehen, da die momentane nutzungsspezifische Reifeklassifizierung die Unterschiede von generativer und vegetativer Abreife nicht bzw. nur unvollständig erfasst. Zum anderen können keine gesicherten Aussagen über die Qualitätsentwicklung unterschiedlicher Abreifetypen getroffen werden.

*Einflussfaktoren auf die Qualitätsentwicklung - umweltbedingte Variabilität*

Neben den genetischen Eigenschaften einer Sorte beeinflussen Umwelteinflüsse als wichtigster Faktor die Entwicklung und Abreife und somit die Ertrags- und Qualitätsparameter von Silomais. Die Umweltbedingungen wirken auf eine Vielzahl von physiologischen Prozessen ein, besonders in sensitiven Phasen der Entwicklung (Struik, 1983a, b, c). In der vegetativen Phase beeinflussen Einstrahlung, Temperatur und Wasserangebot v. a. die morphologische Entwicklung, wie z.B. Blatterscheinungsrate, Blattgröße und Längenwachstum (Bos et al., 2000). Diese wiederum wirken direkt und/oder indirekt auf die Photosyntheseleistung der Pflanze (Teeri et al., 1977; Andrade et al., 1993; Fryer et al., 1995) und beeinflussen somit die Bevorratung von Assimilaten, die in der späteren Entwicklung von

hoher Bedeutung für die Qualität sind (Setter und Flannigan, 1989; Andrade et al., 1999). Witterungsbedingungen, die während der Blüte auf die Maispflanzen einwirken, beeinflussen in hohem Maße die sich daran anschließende generative Entwicklung und determinieren sowohl der Ertrag als auch die Qualität. Unzureichende Befruchtung und/oder Absterben der Kornanlagen verursachen Unterschiede in den Source-to-sink Verhältnissen, die wiederum die TM- und Stärkeakkumulation beeinflussen (Phipps et al., 1984; Uhart und Andrade, 1995; Coors et al., 1997; Borras et al., 2002). Um den Ansprüchen der Silierung zu entsprechen, sollte der TS-Gehalt in der Gesamtpflanze zur Ernte zwischen 30 und 35% liegen (Allen et al., 2003). Höhere Gehalte stehen der Verdichtung und somit der nötigen Sauerstoffverdrängung für die erfolgreiche Milchsäuregärung entgegen (Pahlow et al., 2003), während zu niedrige TS-Gehalte im Erntegut zu TM- und Nährstoffverlusten durch Sickersaft führen (Savoie und Jofriet, 2003). Ebenfalls hat der TS-Gehalt von Maissilage erhebliche Auswirkungen auf die Futteraufnahme von Wiederkäuern. Ältere Untersuchungen belegen eine stetige Zunahme der TM-Aufnahme bei steigenden TS-Gehalten in der Maissilage (Böhm et al., 1983). Dieses gilt jedoch nur bis zu einem TS-Gehalt in der Silage von 30% (Bal et al., 1997; Phipps et al., 2000; Ettle und Schwarz, 2003).

In marginalen Anbaugebieten, wie z. B. in Norddeutschland, determinieren vor allem niedrige Temperaturen während der Kornfüllungsphase gekoppelt mit oftmals niedrigen Einstrahlungsintensitäten und hohen Niederschlägen die Abreife von Silomais. Häufig wird die Siloreife nicht erreicht und somit das genetische Potenzial nicht vollständig ausgenutzt (Herrmann et al., 2005).

*Simulationsmodelle zur Beschreibung der Qualitätsentwicklung*

Aufgrund der Vielzahl von Interaktionen zwischen einzelnen Einflussfaktoren bieten computergestützte Modelle die Möglichkeit, die Auswirkungen der unterschiedlichen Effekte auf die Ertrags- und Qualitätsentwicklung von Pflanzen in ihrer Gesamtheit zu beschreiben. Im Hinblick auf Mais liegt der Fokus hierbei bisher vornehmlich auf der Schätzung bzw. Vorhersage von Ertragsparametern von Körnermais (vgl. Herrmann et al., 2005).

Die Temperatur, die den größten Einfluss auf die Entwicklungsgeschwindigkeit der Pflanze ausübt, wird in den Temperatur-Indices 'growing-degree-days' (GDD) und 'crop-heat-units' (CHU) berücksichtigt. Diese Modelle beruhen auf der funktionalen Beziehung zwischen der

Entwicklungsrate und der Temperatur. Dabei wird eine lineare (GDD) bzw. nicht-lineare (CHU) Beziehung zwischen der Temperatur und der Entwicklungsrate angenommen (Brown und Bootsma, 1993; Bonhomme, 2000). Diese beiden Prognosesysteme werden vor allem in Nordamerika und Frankreich in der Praxis angewendet (u. a. AGPM, 2000).

Am Institut für Pflanzenbau und -züchtung -Grünland und Futterbau/Ökologischer Landbau- der Christian-Albrechts-Universität zu Kiel wurden dynamische Simulationsmodelle entwickelt, die neben der Temperatur weitere wichtige Einflussgrößen wie den Bodenwasserhaushalt und die Einstrahlung berücksichtigen. Die Modelle FOPROQ (FOrage PROduction Quality, Kornher et al., 1991; Herrmann et al., 2005; 2006) und FONSCH (FOrage NonStructural CarboHydrates, Wulfes et al., 1999) kalkulieren unter Berücksichtigung von Managemententscheidungen und Standortbedingungen auf der Basis von genotypischen Parametern sowie Wetterdaten (Tagesdurchschnittstemperatur, Einstrahlung, pflanzenverfügbares Bodenwasser) die Ertrags- und Qualitätsentwicklung von Pflanzen.

In Zusammenarbeit mit dem Deutschen Maiskomitee, dem Deutschen Wetterdienst und den Länderdienststellen der Landwirtschaftskammern wurde im Rahmen des Projektes der 'Regionalen Erntezeitprognose von Silomais' das Modell FOPROQ, welches ursprünglich für Grünlandaufwüchse entwickelt wurde, modifiziert (MAISPROG) und bewies bereits u. a. die Eignung neben dem TM-Ertrag auch den TS- und Stärkegehalt von Silomais zu modellieren (Herrmann et al., 2005). Die Untersuchungen zur Vorhersage des TS-Gehaltes der Gesamtpflanze zeigen gegenüber getesteter Temperatursummenmodelle eine deutliche Steigerung der Prognosegüte (Rath et al., 2005). Das Modell stellt ein verlässliches Werkzeug zur Bestimmung des Erntezeitpunktes dar und wurde bereits in der Praxis implementiert.

Vor diesem Hintergrund können bezüglich des Standes der Forschung zu Fragen der Futterqualität von Silomais folgende Schlüsse gezogen werden:

Die momentane Charakterisierung von Silomaissorten erfolgt neben Anbaueigenschaften weitestgehend über das Ertragspotential. Im Rahmen der Wertprüfung zum Zwecke des Sortenschutzes werden neben den Stärke-, TM- und Energie-Gehalten, eben diese Erträge und weiterhin die Verdaulichkeit (enzymlösliche Substanz (ELOS)) erhoben (Anonymus, 2006d). Landessortenversuche berücksichtigen in ihren Sortenempfehlungen zusätzlich den

Rohproteingehalt (Anonymus, 2006e). Eine weitere Klassifizierung von Maissorten erfolgt über die nutzungsspezifische Reifezahl (Miltner und Rath, 1998), die für Silomais auf Grundlage der TS-Gehalte der Gesamtpflanze festgelegt wird.
Eine weiterführende Quantifizierung der Kohlenhydratfraktionen und dabei v. a. die exakte Bestimmung der Zellwandbestandteile, die als erklärende Größen für den Futterwert gelten, unterbleibt. Auch findet die Qualitätsentwicklung im Laufe der Vegetationsperiode keine Berücksichtigung. Durch das bestehende nutzungsspezifische Klassifizierungssystem werden entkoppelte Abreifen von vegetativen und generativen Pflanzenteilen nicht berücksichtigt, ebenso wenig erfolgt eine differenzierte Beschreibung der Qualitätsentwicklung im Kolben und der Restpflanze.

Zur Qualitätsbestimmung von Silomais ist die Nah-Infrarot-Reflexions-Spektroskopie etabliert, ihr Einsatz in der Praxis wird jedoch auf die oben angesprochenen Standardanalysen wie Stärke, Rohfaser, Rohprotein und Energiebestimmung beschränkt. Die Fähigkeit dieser Methode, exakte Qualitätsanalysen mit minimalem Kosten- und Zeitaufwand durchzuführen, um Silomaisgenotypen anhand der Qualitätsentwicklung im Laufe der Vegetationsperiode zu charakterisieren, wird nicht voll ausgeschöpft. Vor allem die Möglichkeit, eine detaillierte Quantifizierung der Struktur- und Nicht-Struktur-Kohlenhydrate in Kolben und Restpflanze mittels NIRS vorzunehmen, unterbleibt ebenso wie die mögliche Schätzung der Gasbildung zur Ermittlung des energetischen Futterwertes.

Zur Unterstützung verschiedener Managemententscheidungen, wie z. B. der Erntezeitpunktbestimmung, stehen überwiegend Ertragsparameter zur Verfügung, die durch einzelne Qualitätsaspekte, wie TS- und Stärke-Gehalt, ergänzt werden. Die Anwendung adäquater Simulationsmodelle kann den signifikanten Jahreseinfluss auf die Qualitätsentwicklung quantifizieren. Eine Implementierung der Vorhersage von Zellwandbestandteilen (NDF, ADF, Cellulose, Hemicellulose, Lignin), Stärke und wasserlöslichen Kohlenhydraten in bestehende Prognosemodelle kann Entscheidungen auch auf Basis von Qualitätsanforderungen an das Endprodukt Maissilage unterstützen.

Im Rahmen der vorliegenden Arbeit sollte vor diesem Hintergrund zunächst eine Charakterisierung von Silomaisgenotypen anhand futterwertbestimmender Parameter, vor allem der Kohlenhydrat-Fraktionen vorgenommen (i) und der umweltbedingte Einfluss auf die Qualitätsentwicklung im Laufe der Vegetationsperiode quantifiziert (ii) werden. Ein weiteres Ziel war es, die Schätzgenauigkeit der NIRS-Methode zur Bestimmung der Gasbildung zu verbessern (iii).

Die Datengrundlage für die vorgestellten Untersuchungen stellen Feldexperimente dar, die im Rahmen des Projektes der 'Regionalen Erntezeitprognose von Silomais' angelegt wurden. Acht Silomaissorten, die das verfügbare Sortiment hinsichtlich der Reifegruppen, Abreifetypen und voraussichtlicher Inhaltsstoffzusammensetzung abdecken, wurden in einem 3-jährigen Feldversuch auf dem Versuchsgut Hohenschulen der Christian-Albrechts-Universität zu Kiel geprüft. Innerhalb der Vegetationsperiode wurden 6 Ernten (1 vor, 5 nach der Blüte) durchgeführt und die Gehalte an wasserlöslichen Kohlenhydraten, Stärke und Zellwandbestandteilen (NDF, ADF, Hemicellulose, Cellulose, Lignin) in Kolben, Restpflanze und Gesamtpflanze bestimmt. Mittels Varianzanalyse wurde neben dem Einfluss der Sorte auf die Abreife und die Qualitätsentwicklung auch der Jahreseffekt bestimmt. Mit Hilfe der Modelle FOPROQ und FONSCH wurde anschließend geprüft, ob dieser Jahreseffekt durch Unterschiede in der Witterung erklärt und quantifiziert werden kann.

In Kapitel 2 wird der Ansatz dargestellt, die NIRS-Schätzgenauigkeit zur Bestimmung der Gasbildung zu steigern.
Anschließend werden in Kapitel 3 und 4 die genotyp- bzw. umweltbedingten Variationen in den Gehalten an Gerüstsubstanzen und wasserlöslichen Kohlenhydraten quantifiziert, bevor im Kapitel 5 die Ergebnisse abschließend diskutiert werden, wobei ergänzende Untersuchungen zur morphologischen Zusammensetzung, zum TS- und Stärkegehalt vorgestellt werden.

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# Kapitel 2

# Prediction of gas production kinetics of forage maize by near infrared reflectance spectroscopy

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**Abstract**

The in vitro gas production method is widely applied for characterizing the nutritive value of ruminant feeds. Various attempts have been made to predict gas production kinetics of forage maize (*Zea mays* L.) by Near Infrared Reflectance Spectroscopy (NIRS), with only moderate success so far. The aim of the present study was to investigate the causes of this deficiency, which in the case of forage maize has primarily been attributed to the heterogeneity of forage composition, i.e., the concomitance of starchy and fibrous fractions. We therefore investigated the potential of NIRS to predict gas production kinetics separately for maize ear and stover and considered different incubation intervals, which may be related to the fermentation of specific chemical components. A three-year (2001-2003) field experiment was conducted in Northern Germany, where eight silage maize varieties were tested. On six dates within the vegetation period, plants were harvested and separated into ear and stover. In total, 480 samples of ear and 640 samples of stover covering a wide range of maturity stages were available. The samples had been freeze-dried and ground before the spectra were scanned, and calibration and validation subsets were selected. The gas production according to Menke and Steingass (1988) was recorded after 1, 3, 5, 7, 12, 16, 24, 48, and 72 h of incubation. Subsequently, gas production was calculated for ten intervals ranging between 3 and 72 h. Incubation of ear and stover resulted in an average gas production at 72 h incubation of 88.4 ml 200 $mg^{-1}$ dry matter (DM) and 67.9 ml 200 $mg^{-1}$ DM, respectively. Gas volumes recorded in intervals ranged between 2.5 ml 200 $mg^{-1}$ DM in incubation interval 3-5 h of stover and 66.2 ml 200 $mg^{-1}$ DM in interval 5-16 h of ear. NIRS calibration statistics demonstrated satisfactory ability to predict gas production for nearly all intervals analyzed. Best agreement between measured and predicted values was obtained for intervals 3-5 h, 5-16 h, and 16-72 h, with coefficients of determination ($R^2$) exceeding 0.87 and corresponding standard errors of calibration below 3 ml 200 $mg^{-1}$ DM. The calibration equations, however, was less satisfactory to accurately predict gas production of the validation subsets, as indicated by $R^2$-values below 0.81 and high standard errors of prediction, up to 8 ml 200 $mg^{-1}$ DM. Statistics were worse for ear compared to stover samples. Further research is required in order to apply NIRS as a routine method for prediction of gas production kinetics.

**Keywords**

Gas production, Near Infrared Reflectance Spectroscopy (NIRS), silage maize, incubation interval

**Abbreviations**

BBCH: Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie; $CV_M$: coefficient of variation referred to the mean; $CV_{SD}$: coefficient of variation referred to the standard deviation; DM: dry matter; H: Mahalanobis distance; ISI: Infrasoft International; MPLS: Modified Partial Least-Squares method; N: nitrogen; NDF: neutral detergent fiber; NIRS: Near Infrared Reflectance Spectroscopy; R: reflectance; $R^2$: coefficient of determination; SCFA: shorty chain fatty acids; SD: standard deviation; SEC: standard error of calibration; SEV: standard error of validation; VFA: volatile fatty acids.

## 1. Introduction

Maize (*Zea mays* L.) silage is an important component of ruminant diets due to its high energy content and dry matter yield. The most accurate determination of its nutritive value derives from in vivo feeding studies. However, routine analysis is hampered by the need of laboratory facilities and ruminally and/or intestinally cannulated animals, large quantities of feed and high costs. To this account, in vitro and in situ techniques for estimating ruminal digestion of feed were developed, as reviewed by Stern et al. (1997). The nylon bag technique (Mehrez and Ørskov, 1977) is widely considered to be the reference method for evaluating feedstuffs by estimating ruminal nutrient degradation characteristics, as it takes into account digestive processes that occur directly in the rumen. Still, routine or large-scale analysis is limited when only small amounts of sample are available. Furthermore, the time and cost consumption of the technique are substantial.

The in vitro gas production technique, first described by Menke et al. (1979), is based on the empirical relationships between in vivo digestibility and gas production of feedstuffs fermented with rumen liquor to determine their nutritive value. It provides a multitude of information on rumen fermentation kinetics by characterizing the extent and rate of digestion. The accurate quantification of substrate degradation by the in vitro gas production technique is reflected in high correlations between in situ/in vivo parameters and the volume of gas

produced (Blümmel and Ørskov, 1993; Gosselink et al., 2004; De Boever et al., 2005). The gas production and nylon bag methods, however, measure the nutrient degradation in a different manner (Valentin et al., 1999). The significant time and cost savings brought about by automation (Pell and Schofield, 1993; Theodorou et al., 1994; Cone et al., 1994, 1996; Mauricio et al., 2001), low sample size requirements, and a relatively high sample throughput led to increased research in evaluating feed and feed components by the gas production technique. The efficiency of sample evaluation makes the gas production technique applicable to plant breeding programs, where gas measurements can be used as one of the components in a selection matrix (Getachew et al., 2005). Nevertheless, the necessity of keeping cannulated or fistulated animals as donors of rumen fluid together with the know-how of husbandry is a severe disadvantage, although different approaches have been undertaken to replace rumen fluid with alternative media (see review by Mould et al., 2005).

Fermentation kinetics are generally determined by fitting adequate mathematical models to the gas production data (e.g. Mc Donald, 1981; Krishnamoorthy et al., 1991; France et al., 1993), taking into consideration that the overall gas production profile is a result of the fermentation of (i) the water-soluble fraction, (ii) the non-soluble fraction, and (iii) the microbial turnover (Cone et al., 1997). This poses a challenge because of the complexity of the microbial population, which exhibits a large variation in substrate affinity. Furthermore, gas production has to be interpreted as an aggregated value resulting from the fermentation of various substrates at the same time, but at different rates (Sniffen and Robinson, 1987; Chai et al., 2004). Sophisticated model approaches therefore allow for several pools (usually two or three), which represent feed fractions that differ in degradability (Cone et al., 1996; Groot et al., 1996; France et al., 2005).

As an alternative to traditional methods for determining the nutritive value of feedstuffs, the near infrared reflectance spectroscopy (NIRS) technique is widely applied. It is based on the absorbance of light at wavelength regions that relate to chemical components within feeds. This reliable, rapid and non-destructive methodology has shown great potential to predict the chemical composition of feed and feed components without requiring any reagents (Murray, 1993; Stuth et al., 2003). The ability of NIRS to determine various forage quality parameters simultaneously and its capacity for high throughput promoted its routine application in plant breeding programs (Mainka, 1990; Barrière et al., 1997; Jung et al., 1998). Different

approaches have been undertaken to predict by NIRS not only the chemical composition but also biologically meaningful characteristics of feed, where calibration values were derived from in vivo, in situ and in vitro studies (Stuth et al., 2003). The ability to estimate gas production kinetics by NIRS seems to depend on the degree of substrate homogeneity. With respect to maize silage, NIRS prediction of fermentation kinetics has shown only moderate success so far, except for those parameters highly dependent on chemical composition (Lovett et al., 2004). This inability has primarily been attributed to the inhomogeneity of silage maize, which represents a mixture of concentrate and roughage fractions, as characterized by high starch content in grain and mostly fibrous components in the stover.

Accordingly, we hypothesize that the accuracy of NIRS prediction may be improved by estimating gas production separately for fast and slowly available fractions. The objective of our study, therefore, was to explore the potential of NIRS for predicting the gas production of forage maize separately for ear and stover. Instead of fitting models to the measured data and predicting model parameters by NIRS, as commonly applied, we characterized fermentation kinetics by estimating gas production at different incubation intervals, which may be related to the degradation of specific chemical components.

## 2. Materials and Methods

### *2.1 Field experiment*

The study is based on data collected in a 3-year (2001-2003) field experiment conducted at the experimental farm 'Hohenschulen' (53°18'N, 9°58'E, 32 m altitude) of the Faculty of Agricultural and Nutritional Science at the University of Kiel in Northern Germany. The soil type at 'Hohenschulen' can be classified as a pseudogleyic sandy loam. The climate at the experimental site is humid-temperate, with an average annual rainfall of 733 mm and a daily mean temperature of 8.6°C (1974-2005). A one-factorial block design with two replicates (plot size: 90 m²) was used for the field trial, where eight varieties covering a wide range of maturity groups relevant for Germany (early to mid-late) and different maturation types (dry-down, normal and stay-green; high to low harvest index), were investigated. Maize was sown in early May (2$^{nd}$ in 2001, 10$^{th}$ in 2002 and 5$^{th}$ in 2003) in rows 0.75 m apart, with a final plant density of 9-10 plants m$^{-2}$. The amount of applied nitrogen (N) was adjusted to local growth conditions but was limited to a maximum of 150 kg N ha$^{-1}$, split into three dressings: before

planting, first-leaf-stage and 6-8-leaf stage. Phosphorus ($P_2O_5$), potassium ($K_2O$) and magnesium (MgO) were applied at 40, 250, and 30 kg $ha^{-1}$, respectively. Plant protection was conducted according to the codes of 'Good Agricultural Practice in Plant Protection and Fertilization'.

Sampling dates of all varieties were chosen in order to be in line with developmental stages of a mid-early reference cultivar, scheduled to phenological stage of BBCH 32 and an ear dry matter (DM) content of 20, 30, 40, 50, and 55 percent. Sampling dates included collection of plant samples for yield and quality determination. On each sampling date, ten adjacent plants per plot, randomly selected and bordering unharvested rows, were harvested by handclipping close to soil surface. The plants were weighed, separated into ear and stover (including husks), and chopped; a representative sub-sample was oven-dried at 105°C until reaching a constant weight. The samples were reweighed to determine DM content and yield. Another sub-sample of both ear and stover was subsequently stored at -18°C for forage quality determination. After a precautionary freeze-drying process, the samples were ground in a Cyclotec mill (Foss Tecator AB, Höganäs, Sweden) to pass a 1 mm sieve. In total, 480 samples of ears and 640 samples of stover were available for the analysis of quality parameters.

*2.2 NIRS analysis*

All samples available were scanned using a NIRSystems 5000 scanning monochromator (FOSS GmbH, Rellingen, Germany), where software (ISI-version) for data collection and manipulation was supplied by Infrasoft International® (ISI, Port Matilda, PA, USA). Absorbance was recorded as log (1/reflectance) = log (1/R) at 2 nm intervals throughout the near-infrared region (1100-2500 nm) to give a total of 700 data points. Prior to the calibration process samples were checked for erroneous measurements and outliers, using the option 'centre samples' of the ISI® software, which provides a ranking of the spectral data on the basis of the standardized Mahalanobis distance (H) from the average spectrum. Samples with H-values exceeding 3.0 were excluded from the calibration procedure. Calibrations were developed separately for ear and stover. The option 'select samples' on the basis of H-value 0.6 was used to select calibration subsets which represented the whole sample population, while the validation subsets were randomly selected after ranking the spectral data according

to their H distance. Subsets were selected from the pooled 2001 and 2002 data, and were extended by the 2003 samples. Parameters in the following mathematical processing creating the predictive equations were sought through trial and error to minimize the standard errors of calibration (SEC) and to maximise the coefficients of determination ($R^2$). Calibrations were developed by regressing laboratory-determined values against the NIR spectral data, using the Modified Partial Least-Squares (MPLS) method (Shenk and Westerhaus, 1991) with or without scatter correction for particle size. The minimum F statistics for terms included in the equation was 8.0. Spectral data was analyzed using different mathematical treatments as given in Table 1.

Table 1.
Mathematical treatments for calibrating gas production in corresponding incubation intervals.

| incubation interval [h] | mathematical treatments [a,b,c,d] | | scatter correction for particle size | |
|---|---|---|---|---|
| | ear | stover | ear | stover |
| 3-5 | 1, 3, 3, 1 | 2, 4, 4, 1 | yes | yes |
| 3-7 | 1, 5, 5, 1 | 2, 4, 4, 1 | no | no |
| 5-12 | 2, 3, 3, 1 | 2 ,4 ,4 ,1 | yes | no |
| 7-12 | 2, 5, 5, 1 | 2 ,4 ,4 ,1 | yes | no |
| 5-16 | 2, 5, 5, 1 | 2 ,4 ,4 ,1 | yes | no |
| 7-16 | 2, 5, 5, 1 | 2 ,4 ,4 ,1 | no | no |
| 12-16 | 2, 2, 2, 1 | 2 ,4 ,4 ,1 | yes | no |
| 12-24 | 2, 4 ,4 ,1 | 1, 4, 4, 1 | yes | no |
| 24-72 | 2, 4, 4, 1 | 1 ,4 ,4 ,1 | yes | no |
| 16-72 | 2, 2, 2, 1 | 2 ,5 ,5 ,1 | yes | yes |

[a] number of derivate of the log (1/*R*) spectrum
[b] segment of the gap over which the derivative was calculated
[c] numbers of data points used during first smoothing of the spectrum
[d] numbers of data points used during second smoothing of the spectrum

## 2.3 Gas production and statistical analysis

The in vitro gas production was analyzed according to Menke and Steingass (1988). Approximately 200 mg of sample were weighed and placed in graduated glass syringes. Buffer mineral solution was prepared along with modifications proposed by Liu et al. (2002),

including a reduction of $NaHCO_3$ concentration to 33 g $l^{-1}$ and an increase of $(NH_4)HCO_3$ concentration to 6 g $l^{-1}$ in order to prevent a shortage in N during prolonged incubation times. The solution was placed in a water bath at 39°C under continuous $CO_2$ flushing. Rumen fluid was obtained from two ruminally fistulated German Red Pieds steers before morning feeding and was pumped into pre-warmed, insulated flasks using a manually operated vacuum pump. The steers were fed a mixed diet of perennial ryegrass hay and mixed concentrates (2:1, wt/wt). The rumen fluid taken from both animals was mixed, filtered through cheesecloth and flushed with $CO_2$, as all laboratory handlings of rumen fluid were carried out. Subsequently, the rumen fluid was added to the buffered mineral solution (1:2, v/v) and mixed. Thirty milliliters of buffered rumen fluid were pipetted into each syringe, which was placed in a rotor (1 rpm) within an incubator at 39°C. Three blanks containing only 30 ml of medium were included in each assay, as were triplicates of standard hay and standard concentrate obtained from the Institute of Animal Nutrition, Hohenheim University, Germany for controlling the correct progress of analysis. The volume of gas produced by substrate fermentation was recorded after 1, 3, 5, 7, 12, 16, 24, 48, and 72 h of incubation, whereas results refer to the calculated and corrected gas production (in ml) received from 200 mg DM. Dry matter determination was conducted by drying the samples at 105°C until constant weight followed by equilibration in a desiccator. Gas production was then calculated for the incubation intervals 3-5 h, 3-7 h, 5-12 h, 7-12 h, 5-16 h, 7-16 h, 12-16 h, 12-24 h, 24-72 h, and 16-72 h.

Linear regression analysis was performed with Sigma Plot 8.0 (SPSS Inc., 2002) to examine relationships between laboratory-determined and NIRS-predicted values of gas production for selected incubation intervals. The accuracy of the relationships was quantified using coefficient of determination ($R^2$) and slope of the regression curves.

## 3. Results and discussion

### *3.1 Samples*

Due to sequential harvesting, samples were available over a wide range of maturity stages (Table 2). Dry matter content of ear samples varied between 227.4 and 570.5 g $kg^{-1}$ DM in the early varieties and 156.0 to 506.0 g $kg^{-1}$ DM in the mid-late varieties. The stover DM content ranged between 95.7 and 302.7 g $kg^{-1}$ DM for all cultivars tested. Ear and stover

samples displayed a range of carbohydrate contents typically found throughout the vegetation period. In the ear, starch content accumulated up to 613 g $kg^{-1}$ DM, concomitant with a decrease in structural carbohydrates to values as low as 123 g Neutral Detergent Fiber (NDF) $kg^{-1}$ DM. In contrast, NDF contents of stover increased up to 789 g $kg^{-1}$ DM, paralleled by a decline in the content of water-soluble carbohydrates (WSC) down to 7 g $kg^{-1}$ DM (Kruse et al., 2004, 2006).

Table 2.
DM content [g $kg^{-1}$ DM] of ear and stover of tested cultivars (maturity group) at sampling dates; in average of the years 2001-2003.

| Julian Day (sampling date) | Arsenal (early) | | Oldham (early) | | Symphony (early) | | Probat (mid-early) | |
|---|---|---|---|---|---|---|---|---|
| | ear | stover | ear | stover | ear | stover | ear | stover |
| 187 | n.a. | 108.05 | n.a. | 99.40 | n.a. | 114.77 | n.a. | 103.89 |
| 230 | 245.75 | 195.16 | 227.43 | 201.39 | 274.10 | 203.21 | 203.89 | 199.27 |
| 238 | 348.05 | 185.95 | 311.55 | 183.42 | 377.18 | 198.48 | 295.52 | 192.78 |
| 246 | 442.46 | 203.32 | 407.25 | 204.59 | 441.46 | 218.73 | 382.45 | 204.48 |
| 259 | 509.60 | 250.28 | 473.35 | 228.23 | 508.18 | 236.84 | 476.84 | 234.92 |
| 276 | 570.51 | 279.74 | 538.88 | 302.67 | 551.38 | 258.32 | 538.40 | 273.86 |
| Julian Day (sampling date) | Attribut (mid-early) | | Fuego (mid-early) | | Clarica (mid-late) | | Benicia (mid-late) | |
| | ear | stover | ear | stover | ear | stover | ear | stover |
| 187 | n.a. | 112.20 | n.a. | 107.06 | n.a. | 109.44 | n.a. | 95.65 |
| 230 | 2189.95 | 216.92 | 246.86 | 209.84 | 182.47 | 200.23 | 156.03 | 203.66 |
| 238 | 3283.34 | 218.82 | 355.62 | 210.31 | 257.25 | 203.29 | 240.74 | 215.85 |
| 246 | 4385.75 | 220.98 | 448.40 | 209.47 | 346.02 | 206.28 | 341.92 | 210.98 |
| 259 | 5480.43 | 247.13 | 517.07 | 221.06 | 423.72 | 219.46 | 429.80 | 224.59 |
| 276 | 5550.13 | 282.18 | 568.58 | 243.67 | 505.98 | 231.01 | 498.87 | 230.69 |

n.a.: data not available

*3.2 Gas production in total*

Microbial biomass, gases ($CO_2$, $CH_4$ and traces of $H_2$), and volatile fatty acids (VFA) are the main fermentation outputs. Furthermore, neutralization of VFA by the incubation buffer is an additional source of $CO_2$ release (Beuvink and Spoelstra, 1992; Blümmel and Ørskov, 1993; Getachew et al., 1998), while gas volume produced from protein is small and that of fat fermentation is negligible (Menke and Steingass, 1988; Getachew et al., 1998). The overall gas production is basically the result of fermentation of carbohydrates to acetate, propionate and butyrate (Beuvink and Spoelstra, 1992; Blümmel and Ørskov, 1993).

In the present study, a 72-h incubation of ear and stover samples resulted in a mean total gas production of 88.4 ml 200 $mg^{-1}$ DM and 67.9 ml 200 $mg^{-1}$ DM, respectively (data not presented). Compared to findings reported in literature, our gas production values are substantially higher. DePeters et al. (2003), for instance, observed 69.6 ml 200 $mg^{-1}$ DM gas produced after a 72-h incubation of whole-corn grain samples. Tolera et al. (1998) found 44.5 to 49.9 ml 200 $mg^{-1}$ DM for maize stover, but their crop was further advanced in maturity compared to the maize used in our experiment. Several other studies provide potential gas production values, which were obtained by fitting a mathematical model to the measured data. Deavillle and Givens (2001) reported potential gas production of maize silages (DM content 210-380 g $kg^{-1}$) as varying between 210 and 239 ml $g^{-1}$ DM (equiv. 42-48 ml 200 $mg^{-1}$ DM) in a 72-h incubation experiment. Getachew et al. (2004) estimated 59.3 ml 200 $mg^{-1}$ DM potential gas production of corn silage, and 75.6 to 78.4 ml 200 $mg^{-1}$ DM of corn grain and hominy, respectively. As with the aforementioned studies, their findings were based on a 72-h incubation, while Mauricio et al. (2001) estimated 51 ml 200 $mg^{-1}$ DM potential gas production of maize stover silage for a 96-h incubation.

A direct comparison with our findings is hampered for several reasons. First of all, few investigations analyzed ear and stover separately. Fermentation characteristics of whole crop samples, however, may differ substantially from those solely involving starchy ear or fibrous stover samples. Additional factors accounting for differences in gas production pertain to sample preparation and the techniques applied for measuring gas production. The impact of the drying method on the chemical composition of samples has been the subject of various studies (e.g. Cone et al., 1996; Lowman et al., 2002; Colabro et al., 2005), which have shown that the low enzyme activity associated with freeze-drying makes it an effective and non-destructive technique. Drying, however, may result in an overestimation of fermentability, which must be taken into account when predicting forage nutritional parameters from gas production (Rymer et al., 2005). Just as the drying process can alter chemical composition, so can sample grinding influence microbial attachment to feed particles and, therefore, modify gas production volumes. Lowman et al. (2002) found higher gas production volumes in dried and ground samples of maize silage compared to fresh, unground material. They concluded that the discrepancy may be caused by the damage and alteration of fresh samples within the drying and grinding process, facilitating microbial

attachment and leading to increased gas production. In contrast, Colabro et al. (2005) attributed the differences in gas production to nutrient availability and the ease of colonization rather than particle size.

In the present project, fresh material of ear and stover, which was only subjected to freeze-drying and grinding, was used to determine gas production kinetics. We may thus assume that the material underwent neither changes in chemical composition, e.g. through fermentation of soluble carbohydrates by lactic acid bacteria during the ensiling process, nor volatilization during oven-drying processes. This may in part explain the higher gas production observed.

*3.3 Gas production in incubation intervals*

The maize samples covered a wide range of maturity stages and, by extension, chemical compositions, which influenced gas production of the intervals investigated. Incubation of ear samples resulted in gas volumes ranging between 2.46 ml 200 $mg^{-1}$ DM for the interval 3-5 h and 66.19 ml 200 $mg^{-1}$ DM for the interval 5-16 h (see Table 3). Stover was characterized by a substantially lower gas production, ranging from 1.40 to 44.79 ml 200 $mg^{-1}$ DM in incubation intervals 3-5 and 16-72 h, respectively.

Differences in the fermentation characteristics of the various feed components arise from the complexity of the microbial population and its widely varying substrate affinity. Gas production is a result of various chemical components being fermented simultaneously, but at different rates (Sniffen and Robinson, 1987; Chai et al., 2004). According to Cone et al. (1997), the gas production profile can be divided into three sub-curves describing the gas production caused by fermentation of (i) the water-soluble fraction (i.e. water-soluble carbohydrates and most of the proteins), (ii) the non-soluble fraction (starch and NDF), and (iii) the microbial turnover. The initial gas production is mainly caused by the fermentation of the soluble-fraction, for instance of water-soluble carbohydrates (Sniffen and Robinson, 1987; Cone et al., 1996; Chai et al., 2004), which is assumed to be completed within 3 hours of incubation (Cone et al., 1996). In the present study, gas produced within the first 3 hours was not taken into consideration due to the lag-phase required for microbial colonization of feed particles, which is estimated to range between 1 and 4 hours (Deaville and Givens, 2001; Lovett et al., 2004). Gas production of interval 3-5 h varied between 2.46-19.06 ml 200 $mg^{-1}$ DM in ear

and 1.4-12.71 ml 200 $mg^{-1}$ DM in stover. Data for the year 2001, which were analyzed completely, indicate a relatively close relationship between stage of maturity and gas produced in interval 3-5 h. For both ear and stover, gas production decreased with advancing maturity (data not presented). This was paralleled by a steady decrease of WSC content in ear and stover, whereas NDF content declined in ear but increased in stover, and starch content showed a strong rise in ear (Kruse et al., 2004, 2006). We therefore conclude that the differences in gas production observed in interval 3-5 h can mainly be attributed to the availability of soluble components, especially of water-soluble carbohydrates.

Fermentation of the soluble fraction is followed by starch degradation. The majority of starch fermentation occurs after 6 h of incubation and is completed within 24 h (Chai et al., 2004). However, Chai et al. (2004) also showed data indicating a stronger relationship between starch degradation and gas production in interval 6-16 h compared to intervals 6-24 h and 6-32 h. This is supported by our own data, which display largest differences in mean gas production between ear and stover for intervals 5-16 h (26.6 ml) and 7-16 h (23.7 ml), see Table 3. Furthermore, ear gas production in interval 5-16 h increased with advancing maturity (data not presented). According to DePeters et al. (2003), starch degradation is half-completed after 5.5 to 6.8 hours, depending on sample processing. However, it may continue up to 32 h or even longer (DePeters et al., 2003; Chai et al., 2004). These findings are in good agreement with Getachew et al. (2004), who found non-fiber carbohydrate content to be more closely related to gas production after 24 h and 48 h than to gas produced after 6 h incubation.

In addition, Cone et al. (1994) mentioned fermentation of easily fermentable cell wall tissues between 5 and 15 h of incubation, while gas production after 15 h was assumed to be related only to the degradation of less-fermentable cell wall components or microbial turnover. Typically, the gas production rate slows down in later phases of fermentation, as in our study, where the average gas production of interval 16-72 h was 26.3 ml 200 $mg^{-1}$ DM in ear and 29.9 ml 200 $mg^{-1}$ DM in stover (Table 3).

*3.4 NIRS determination of gas production*

The gas production technique (Menke and Steingass, 1988) requires long incubation intervals and fistulated ruminants but provides meaningful biological parameters of forage quality.

Sample throughput might be enhanced substantially if NIRS proves to be a viable method for estimating gas production. In the present study, calibration and validation subsets for ear and stover were selected by pooling the spectral data of the first two experimental years (2001, 2002) and extending the obtained subsets with samples from 2003. This extension made up 10-20% of analyzed ear and stover calibration samples, whereas the validation subsets both consisted of 50% samples originating from 2003. A total of 88 representative samples of ear and 210 samples of stover were selected for calibrating gas production in different incubation intervals. For validation, 40 samples each of ear and stover were selected. Calibrations were developed separately for each of the 10 incubation intervals by using coefficient of determination ($R^2$) and standard error of calibration (SEC) as criteria for useful equations. Missing values and outliers eliminated during the mathematical calibration process decreased the total number of samples finally included in the NIRS analysis. Depending on incubation interval, 66 to 71 measurements of ear samples and 165 to 177 measurements of stover samples were included in the calibration, while 36 measurements for stover and 35 for ear were considered when validating the prediction equation (Tables 3 and 4).

Calibration statistics exhibited a satisfactory ability to estimate gas production for different incubation intervals, except for intervals 12-16 h in ear and stover, and interval 12-24 h in stover, as indicated by coefficients of determination ($R^2$) ranging between 0.83 and 0.96 for ear and between 0.83 and 0.93 for stover (Table 3). Standard error of calibration (SEC) showed large differences between intervals, with values varying from 1.07 to 5.39 in ear and from 0.71 to 2.25 in stover.

Table 3.

Calibration statistics of the gas production in different incubation intervals.

| incubation interval [h] | n (1) | | range (2) | | | | mean (3) | | S.D. (4) | | SEC (5) | | $R^2$ (6) | | $CV_M$ (7) | | $CV_{SD}$ (8) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ear | stover | ear | | stover | | ear | stover | ear | stover | ear | stover | ear | stover | ear | stover | ear | stover |
| 3-5 | 63 | 173 | 2.5 | 19.1 | 2.0 | 12.7 | 7.5 | 6.5 | 4.29 | 2.99 | 1.07 | 0.87 | 0.94 | 0.92 | 14.36 | 13.41 | 24.94 | 29.10 |
| 3-7 | 69 | 170 | 5.9 | 40.1 | 3.5 | 22.8 | 17.3 | 13.2 | 8.90 | 5.13 | 3.71 | 1.35 | 0.83 | 0.93 | 21.51 | 10.23 | 41.69 | 26.32 |
| 5-12 | 65 | 169 | 17.5 | 55.3 | 8.3 | 31.3 | 37.8 | 19.5 | 9.23 | 5.61 | 3.07 | 1.83 | 0.89 | 0.89 | 8.12 | 9.38 | 33.26 | 32.62 |
| 7-12 | 71 | 170 | 13.8 | 42.4 | 5.5 | 19.5 | 27.9 | 12.5 | 7.06 | 3.12 | 2.97 | 1.19 | 0.83 | 0.86 | 10.64 | 9.49 | 42.07 | 38.14 |
| 5-16 | 67 | 168 | 30.6 | 66.2 | 12.5 | 42.9 | 52.3 | 25.7 | 9.20 | 6.36 | 1.81 | 1.82 | 0.96 | 0.92 | 3.46 | 7.07 | 19.67 | 28.62 |
| 7-16 | 70 | 171 | 20.1 | 58.3 | 9.7 | 31.3 | 42.5 | 18.8 | 9.41 | 4.24 | 2.93 | 1.68 | 0.90 | 0.83 | 6.90 | 8.96 | 31.14 | 39.62 |
| 12-16 | 71 | 161 | 4.9 | 28.7 | 2.8 | 10.8 | 14.7 | 6.1 | 6.03 | 1.36 | 5.39 | 0.71 | 0.20 | 0.66 | 36.62 | 11.56 | 89.39 | 52.21 |
| 12-24 | 66 | 170 | 12.5 | 50.8 | 8.7 | 23.5 | 25.8 | 15.7 | 10.56 | 3.28 | 3.88 | 2.12 | 0.85 | 0.62 | 15.03 | 13.51 | 36.74 | 64.63 |
| 24-72 | 67 | 168 | 9.8 | 26.7 | 9.9 | 34.1 | 15.2 | 20.6 | 3.60 | 5.58 | 1.24 | 2.25 | 0.88 | 0.83 | 8.16 | 10.92 | 34.44 | 40.32 |
| 16-72 | 69 | 162 | 12.1 | 56.6 | 15.7 | 44.8 | 26.3 | 29.9 | 8.88 | 7.43 | 3.17 | 2.20 | 0.87 | 0.91 | 12.06 | 7.36 | 35.70 | 29.61 |

(1): Number of samples included in the calibration

(2): Minimum- and maximum-volume of gas produced [in ml 200 $mg^{-1}$DM]

(3): Mean volume of gas produced [in ml 200 $mg^{-1}$DM]

(4): Standard deviation of the laboratory–determined values

(5): Standard error of calibration

(6): Coefficient of determination; relationship between NIRS- and laboratory-determined values

(7): Variation coefficient referred to the mean ( $CV_M = SEC \cdot 100 / mean$ )

(8): Variation coefficient referred to the SD of the reference method ( $CV_{SD} = SEC \cdot 100 / SD$ )

The SEC statistic, however, has only limited suitability for comparing the accuracy of NIRS prediction across intervals, if those are characterized by large differences in mean gas production. A better tool for evaluating NIRS calibration performance across intervals is the variation coefficient referring to the mean ($CV_M = SEC \cdot 100 / mean$) (Hruschka, 1987; Clark et al., 1989). Additionally, we calculated the variation coefficient referring to the standard deviation (SD) ($CV_{SD} = SEC \cdot 100 / SD$) in order to assess the suitability of the gas production technique for reliable NIRS calibration, as suggested by Murray (1986). The SEC should not exceed 10% of the mean (determined by reference method) (Hruschka, 1987) and should be lower than 30% of the SD of the reference method (Murray, 1986).

The $CV_M$ values of ear and stover were within the 10%-margin for intervals 5-12 h, 5-16 h, 7-16 h, and 24-72 h. For $CV_{SD}$, only intervals 3-5 h and 5-16 h stayed below the 30% threshold in ear and stover, while intervals 3-7 h and 16-72 h met the criterion for stover, but not for ear (Table 3). Taking all criteria into consideration, the best agreement between laboratory-determined and NIRS-predicted gas production for both ear and stover, was obtained for intervals 3-5 h, 5-16 h, and 16-72 h, with $R^2$ values ranging between 0.87 and 0.96, $CV_M$ values from 3.46 to 14.36, and $CV_{SD}$ between 19.67 and 35.70. Figure 1 displays in detail the results of NIRS calibration for intervals 3-5 h, 5-16 h, and 16-72 h. The more accurate the calibration equation, the more closely all points cluster near the bisecting line (dashed line). Regression slopes below 1.0 and intercepts differing from zero observed for all three intervals considered indicate a slight underestimation of laboratory-determined values in the upper range and an overestimation in the lower range. There are neither considerable outliers present in the data sets nor any obvious differences in prediction accuracy among years to explain the systematic error, which became most apparent in interval 16-72 h of ear. Rather, we find a relatively high variation around the regression line. In contrast to NIRS calibration, validation statistics demonstrate a less successful prediction of gas production. For all intervals investigated the coefficients of variation were below 0.81 and the SEV values of ear varied between 2.93 and 8.02 and of stover between 0.80 and 4.07, which demonstrates that the calibration equations lack robustness. The difficulties in NIRS prediction seem to be most apparent in the ear samples, as indicated by the ratio of SEV to SEC varying between 1.0 to 1.9 for stover (in interval 12-24 h the ratio even was below 1.0), whereas the ratio ranged between 1.3 and 3.3 for the ear samples.

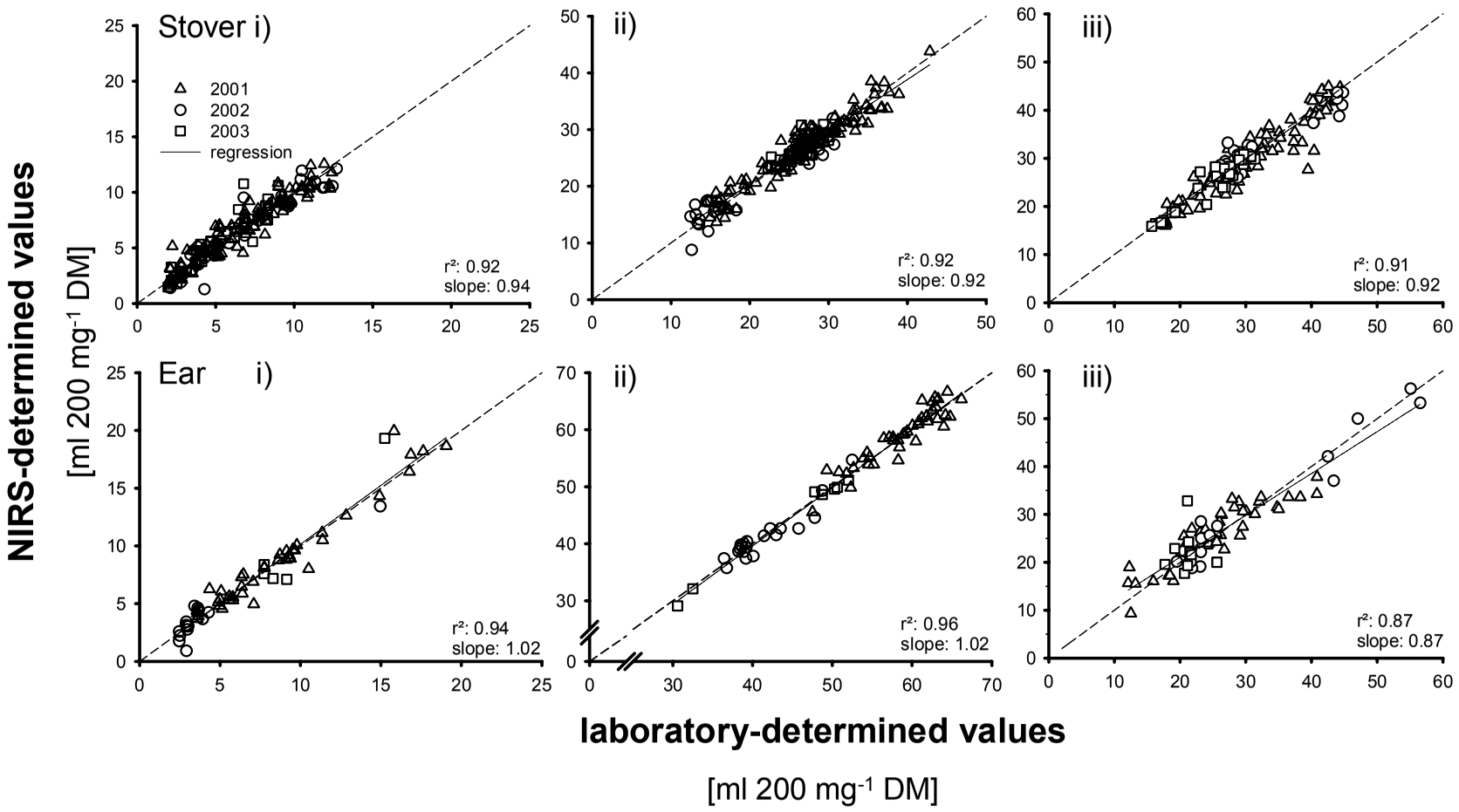


Figure 1.

Relationship for calibration samples between NIRS- and laboratory-determined gas production volumes of intervals (i) 3-5 h, (ii) 5-16 h, and (iii) 16-72 h.

The difficulties in NIRS prediction seem to be most apparent in the ear samples, as indicated by the ratio of SEV to SEC varying between 1.0 to 1.9 for stover (in interval 12-24 h the ratio even was below 1.0), whereas the ratio ranged between 1.3 and 3.3 for the ear samples. This is illustrated by Figure 2, which presents the laboratory-determined and NIRS-predicted gas production for intervals 3-5 h, 5-16 h, and 16-72 h. We find consistently lower slopes of regression and larger variations around the regression lines for ear compared to stover.

Several factors may influence the success of NIRS equation development, such as the accuracy of laboratory determined data and the errors related to NIRS calibration. Crucial to improving NIRS predictions is the degree of accuracy associated with the reference method, since the validity of NIRS-predicted data will never be better than the reference methods used for establishing the NIRS equations (Coates, 2002). Parameters measured by biological methods such as the gas production technique are subject to a multitude of error sources, which may increase variability. With respect to the accuracy of gas production measurements, we must differentiate between repeatability within a run and reproducibility between runs on different days. While Getachew et al. (2002) reported a high degree of reproducibility, Van Laar et al. (2006) pointed out considerable differences among incubation runs. Pell and Schofield (1993), evaluating the variation of measurements over incubation time, detected a slight decline in repeatability. Reproducibility among runs on different days was similar in pattern, but had a larger variation during early incubation. This is in accordance with Blümmel and Becker (1997), who found the reproducibility of the gas production rate to be lower than that of the total amount of gas produced. In the present study, however, no clear trend in prediction accuracy over incubation time became evident. Another potential reason for poor NIRS statistics refers to unexplained or systematic errors (bias), which may be introduced when NIRS equations are used to predict the chemical composition or biological parameters of samples that are not properly represented in the calibration data set (Smith and Kearney, 2000). This does not apply to the present study, where calibration and validation subsets originated from the same field experiment. Furthermore, the range of gas production volumes was somewhat larger in the calibration compared to the validation subset (Tables 3 and 4). It may therefore be assumed that all sources of variation encountered in data sampling and routine analysis were covered in the calibration as well as in the validation subsets.

Table 4.

Validation statistics of the gas production in different incubation intervals.

| incubation interval [h] | n (1) | | range (2) | | | | mean (3) | | SEV (4) | | $R^2$ (5) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ear | stover | ear | | stover | | ear | stover | ear | stover | ear | stover |
| 3-5 | 35 | 36 | 1.9 | 19.7 | 1.9 | 13.3 | 10.3 | 7.0 | 3.58 | 1.55 | 0.64 | 0.69 |
| 3-7 | 35 | 36 | 5.4 | 34.7 | 4.9 | 22.5 | 21.7 | 13.8 | 7.47 | 1.75 | 0.51 | 0.81 |
| 5-12 | 35 | 36 | 23.4 | 45.2 | 8.3 | 26.3 | 32.6 | 19.1 | 5.56 | 2.23 | 0.36 | 0.71 |
| 7-12 | 35 | 36 | 13.8 | 32.2 | 5.3 | 16.1 | 21.2 | 12.3 | 6.09 | 1.22 | 0.21 | 0.78 |
| 5-16 | 35 | 36 | 28.8 | 59.0 | 12.1 | 33.3 | 41.4 | 25.3 | 6.06 | 2.67 | 0.46 | 0.69 |
| 7-16 | 35 | 36 | 17.7 | 51.8 | 9.3 | 23.6 | 30.0 | 18.5 | 8.02 | 1.74 | 0.40 | 0.74 |
| 12-16 | 35 | 36 | 1.4 | 24.1 | 3.5 | 8.0 | 8.8 | 6.3 | 6.94 | 0.80 | 0.12 | 0.64 |
| 12-24 | 35 | 36 | 11.8 | 47.5 | 7.7 | 26.0 | 18.2 | 14.8 | 6.03 | 2.05 | 0.51 | 0.53 |
| 24-72 | 35 | 36 | 9.2 | 22.4 | 11.0 | 26.8 | 12.4 | 18.0 | 2.93 | 3.09 | 0.12 | 0.47 |
| 16-72 | 35 | 36 | 11.1 | 45.8 | 16.8 | 40.1 | 21.7 | 26.6 | 5.92 | 4.07 | 0.35 | 0.46 |

(1): Numbers of samples included in the validation subset
(2): Minimum- and maximum-volume of gas produced [ml 200 $mg^{-1}$ DM]
(3): Mean volume of gas produced [in ml 200 $mg^{-1}$ DM]
(4): Standard error of validation
(5): Coefficient of determination; relationship between NIRS- and laboratory-determined values

Even though standard sample selection and analysis procedures were applied, and an adequate representation of the sample populations by the calibration subsets may be assumed, another source of error in NIRS prediction has to be taken into consideration. Generally, it is presumed that errors in NIRS prediction are randomly distributed across all samples. However, there remains the possibility of significant non-random errors associated with the population structure, e.g. year, field replicate, and cultivar (Buxton and Mertens, 1991). These errors can be detected even when the calibration subsets are balanced with respect to *a priori* knowledge of the sample structure (Smith and Kearny, 2000). Due to a lack of information concerning the impact of sample structure on NIRS prediction accuracy in our study, we cannot exclude non-random errors. Aside from reference methods and data structure, the inhomogeneity of maize, which contains both fibrous and starchy fractions, was addressed by Lovett et al. (2004) as a further possible reason for errors in estimating gas production kinetics. The authors evaluated the potential of NIRS to predict gas production kinetics of maize silage by fitting an appropriate nonlinear model to the measured data and subsequently estimated model parameters by NIRS. Calibration statistics demonstrated large problems in predicting the modeled gas production kinetics.

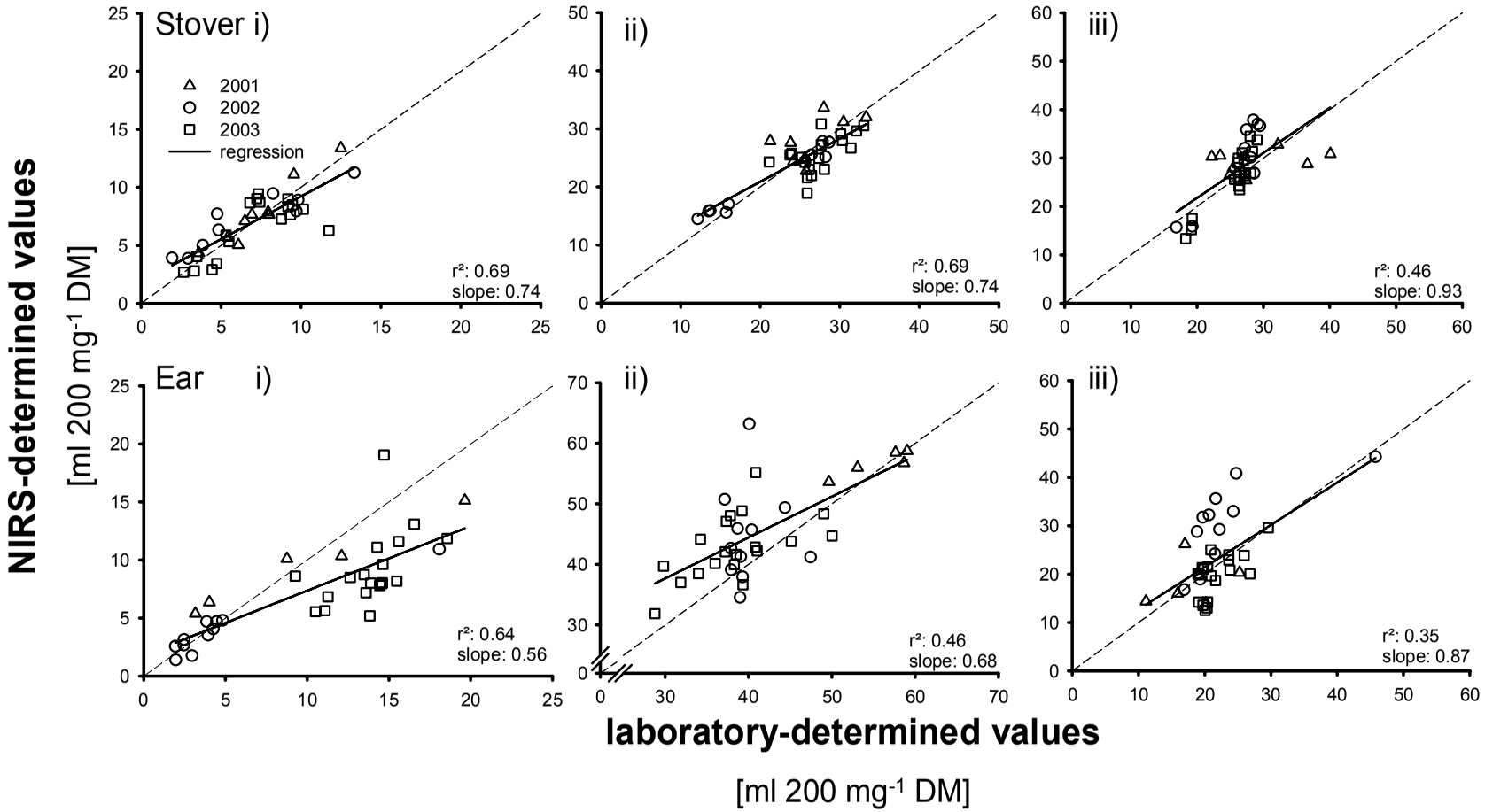


Figure 2.

Relationship for validation samples between NIRS- and laboratory-determined gas production volumes of intervals (i) 3-5 h, (ii) 5-16 h, and (iii) 16-72 h.

We thus hypothesized that prediction accuracy might be increased by using more homogeneous sample populations and developed calibration equations separately for ear and stover. Furthermore, we assumed that the degradation of the different chemical constituents, e.g. starch and water-soluble carbohydrates, could be assigned to defined incubation intervals. This approach, however, encounters some difficulties. The time-dependent fermentation of different substrates overlapped more than previously supposed; in addition, the fermentation intensity of the different incubation intervals varied substantially, as indicated in our study by high standard deviations of gas volumes measured (Table 3), which may be attributed to the wide range of samples collected from early stages to nearly physiological maturity. In case of maize starch, for instance, maturity has a substantial impact on the ratio of vitreous to floury endosperm. Generally, kernel vitreousness increases with advancing maturity (Correa et al., 2002). Since vitreousness is negatively correlated with ruminal starch availability, maturity will influence gas production directly, as indicated by Getachew et al. (2005). Indirectly, the maturity-dependent changes in substrate composition and availability determine the short-chain fatty acid (SCFA) profile, which in turn influences gas volume produced (Blümmel et al., 1999). It is well known that feeds rich in non-structural carbohydrates produce high amounts of propionate, resulting in lower yields of gas volumes (Beuvink and Spoelstra, 1992). Lovett et al. (2004) regard this indirect effect of maturity on the SCFA profile as the main cause for the inability of NIRS to predict gas production kinetics. Moreover, most plant carbohydrate types absorb in similar spectral regions (Deaville and Givens, 1998), which also may limit NIRS prediction of gas production for different incubation intervals. Taken together, our results indicate that further improvement is required for reliable prediction of gas production by NIRS.

**4. Conclusions**

The use of near-infrared reflectance spectroscopy (NIRS) for predicting gas production kinetics of silage maize has thus far been of limited validity, which can be primarily ascribed to the inhomogeneity in substrate composition. Our attempts to increase homogeneity by (i) developing calibration equations separately for ear and stover and (ii) considering different incubation intervals showed promising results for NIRS calibration, while validation statistics were poor. Improving NIRS prediction of gas production kinetics remains a considerable

challenge. On the one hand, a large variation in sample population is required in order to obtain representative calibrations, while on the other hand variation in substrate composition and availability caused by maturation seems to be a chief cause for the limited potential of NIRS to predict gas production kinetics.

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# Kapitel 3

# Characterization of silage maize hybrids with fiber fraction development during vegetation period-

## *Genotypic impacts and weather-based modeling*

Sandra Kruse, Antje Herrmann, Alois Kornher, Friedhelm Taube

**Abstract**

The cell wall content of crops may limit digestibility and forage intake in ruminant nutrition and methane output in anaerobic digestion. Comparative studies on the impact of hybrid and environment on the content of cell wall constituents of silage maize are scarce. The objectives of this study therefore were to examine the contribution of genotype and environment on seasonal changes of fiber components (neutral detergent fiber (NDF), acid detergent fiber (ADF), hemicellulose, cellulose, lignin) of stover, ear, and whole crop.

Furthermore, the study intended to evaluate, if the impact of weather conditions (temperature, radiation, soil water availability) could be quantified by the dynamic FOPROQ model. A three-year field experiment (2001-2003) was conducted in Northern Germany to evaluate differences in fiber content among commercial silage maize varieties across different growing conditions, and to serve as calibration data base for modeling. Eight varieties covering a wide range of maturity groups (early to mid-late) and different maturation types (dry-down, normal, stay-green) were investigated. At six dates throughout the vegetation period plants were harvested, separated into ear and stover, and freeze-dried for subsequent determination of NDF, ADF, cellulose, hemicellulose, and lignin. Few interactions of variety within maturity group x harvest date were observed, which furthermore occurred at early growth stages only. Differentiation was more evident for maturity groups after grain set, with a maximum difference of 58.1 g NDF $kg^{-1}$ DM, 43.0 g ADF $kg^{-1}$ DM, 37.5 g cellulose $kg^{-1}$ DM, and 15.2 g hemicellulose $kg^{-1}$ DM for the whole crop, but diminished with advancing maturity. The model proved suitable for describing seasonal changes of fiber components, with RMSE values below 22.03 g NDF $kg^{-1}$ DM, 15.09 g ADF $kg^{-1}$ DM, 14.30 g cellulose $kg^{-1}$ DM, and 11.75 g hemicellulose $kg^{-1}$ DM for the whole-crop of selected cultivars. Model calibration revealed quality change to be mainly driven by temperature and radiation, whereas soil water availability had a negligible impact. A 30-year simulation study documented a considerable environmentally caused variation of fiber components, with coefficients of variation ranging between 3.6% (hemicellulose) and 8.9% (cellulose). Compared to the environmental conditions, the impact of genotype seems marginal.

**Key words**

cell wall constituents, genotype, environmental conditions, model, simulation study

**Abbreviations**

ADF: acid detergent fiber; BBCH: Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie; *c*: determines the inclination of the threshold response curve; DM: dry matter; ISI: Infrasoft International; MPLS: Modified Partial Least-Squares method; NDF: neutral detergent fiber; NIRS: Near Infrared Reflectance Spectroscopy; $m_c$: constant in soil moisture change function; $Q_n$: minimum content of fiber component at the onset of growth; $Q_x$: maximum content at maturity; $R^2$: coefficient of determination; $r_c$ : constant of radiation change function; $R_{th}$ : radiation threshold in radiation change function; RMSE: root mean squared error; $t_c$ : constant of temperature change function; $T_{th}$ : temperature threshold in temperature change function; *v* : determines the inflexion point of the threshold response curve.

## 1. Introduction

Silage maize (*Zea mays* L.) is a key component of ruminant diets due to its high yield and energy content, and in recent years this crop additionally gained importance as substrate for biogas production, especially in Germany. For ruminant nutrition as well as for biogas production it is essential to harvest the crop at the optimum developmental stage, because forage quality and methane yield may change substantially with advancing maturity (Filya, 2004; Heiermann and Plöchl, 2004). Cell wall concentration and composition, as well as their degradability, are considered to be major limitations of forage digestibility (Jung and Deetz, 1993; Jensen et al., 2005), and to influence anaerobic digestion (Eder et al., 2006). Accordingly, the quantity of cell wall fractions may serve as an indicator of quality changes in forage maize, especially for maize stover (Cone and Engles, 1993; Verbic et al., 1995).

Genotype and environmental conditions are known as potential influencing factors of the content of cell wall fractions (Struik et al., 1985; Hunt et al., 1993). Breeding for adaptation to cool temperate growing regions resulted in changes in yield physiology, morphology, stress tolerance (Tollenaar and Wu, 1999; Frei, 2000), and in genetic variation for cell wall content and quality (Struik, 1985; Deinum, 1988; Jung and Buxton, 1994). Efforts to improve stover quality, as for instance by the introduction of stay-green hybrids, so far have shown little progress (Ettle and Schwarz, 2003). Brown midrib hybrids, which are characterized by higher cell wall digestibility (Barrière et al., 2003), are of no importance on the European market.

Environmental conditions may affect the content of cell wall fractions directly and/or indirectly, if plant morphology, e.g. the ear/stover ratio, is modified. The study of Struik et al. (1985)

documented the impact of temperature on cell wall content in different development phases. Consistently, lower cell wall concentrations were reported for hot and/or dry growing seasons compared to cool and/or wet conditions (Cox et al. 1994; Allen et al. 1991; Wiersma et al., 1993), which may be due to the accumulation of water-soluble carbohydrates (Crasta et al., 1997). So far, however, no study has comprehensively investigated the importance of both, genotype and environmental impact factors on the seasonal changes of cell wall fractions.

The use of appropriate models might facilitate to quantify the environmental impact on fiber content and provide tools for predicting cell wall content and composition. Crasta et al. (1997) applied multiple regression using temperature, soil water availability, and their interaction as independent variables for estimating whole crop contents of neutral detergent fiber (NDF), acid detergent fiber (ADF), and lignin. This approach, however, was not able to explain more than 60 percent of the total variability. In a similar work Darby and Lauer (2002) related NDF and ADF contents of whole crop and stover to growing degree units. The study resulted in promising relationships, but was based on 2-years only, which both had above average temperature. The dynamic, weather-based FOPROQ model (Kornher et al., 1991), originally developed for grassland, considers the influence of environmental conditions, e.g. air temperature, radiation and plant-available soil water, and of crop characteristics and management inputs on development of yield and forage quality. Recently, the model has been successfully introduced as a tool for harvest time prognosis in forage maize (Herrmann et al., 2005; Rath et al., 2005). In its current version prognosis is restricted to whole crop dry matter content. Extending the model applicability to the content of cell wall fractions would be useful from a scientific and practical point of view.

The first objective of the present study therefore was to investigate the contribution of genotype and environmental conditions on seasonal changes of cell wall fractions (NDF, ADF, hemicellulose, cellulose, lignin) of stover, ear, and whole crop. The second objective was to evaluate, if the impact of weather conditions, e.g. temperature, precipitation, and radiation, on changes of fiber components period can be quantified by the FOPROQ model.

## 2. Materials and Methods

### *2.1 Experimental design and trial management*

The study is based on data collected in a 3-year (2001-2003) field experiment conducted at the experimental farm 'Hohenschulen' (53°18'N, 9°58'E, 32 m altitude) of the Faculty of Agricultural and Nutritional Science at the University of Kiel in Northern Germany. The soil type at 'Hohenschulen' can be classified as pseudogleyic sandy loam. The prevailing climate at the experimental site is humid-temperate, with an average annual precipitation of 759 mm and daily mean temperature of 8.7 °C (1974-2005). Weather conditions differed considerably among the experimental years, see Tab. 1. While in 2002 and 2003 mean temperature of the maize vegetation period (1$^{st}$ May to 30$^{th}$ September) exceeded the long-term average substantially by up to 2 °C, temperature was average for 2001. Precipitation was exceptionally low in 2003 with only 63% of the long-term amount, whereas the previous years had above average rainfall.

A one-factorial block design with two replicates (plot size: 90 m²) was used for the field trial, where eight hybrids (Arsenal, Oldham, Symphony, Probat, Attribut, Fuego, Clarica, Benicia), covering a wide range of maturity groups relevant for Germany (early to mid-late) and different maturation types (dry-down, normal and stay-green; high to low harvest index), were investigated. Maize was sown in early May in rows 0.75 m apart, with a final plant density of 9-10 plants $m^{-2}$. Plots received an annual application of 150 kg nitrogen (N) $ha^{-1}$, split into three dressings: before planting, first-leaf-stage and 6-8-leaf stage. Phosphorous ($P_2O_5$), potassium ($K_2O$) and magnesium (MgO) were applied at 40, 250, and 30 kg $ha^{-1}$, respectively. Plant protection was conducted according to the codes of 'Good Agricultural Practice in Plant Protection and Fertilization'.

Crop samples were taken on six dates, which were chosen to be in line with developmental stages of a reference hybrid (Probat, mid-early maturity group), scheduled to phenological stage of BBCH 32 (Meier, 2001) and ear dry matter (DM) content of 20, 30, 40, 50, and 55 percent, respectively. The sampling schedule included the recording of crop phenology on each date, the occurrence of key growth stages, e.g. tasseling and silking, and the collection of plant samples for yield and quality determination.

Ten consecutive plants, randomly assigned to a row section bordered by un-harvested rows, were handclipped near soil surface. The plants were weighed, separated into ear and stover (including husks), and chopped. Representative sub-samples of ear and stover were oven-

dried at 105°C until constant weight to obtain DM content and yield. Additional sub-samples of ear and stover were stored at -18°C for quality determination. After freeze-drying the samples were at first pre-ground in a rotor beater mill to pass a 4 mm sieve (Retsch GmbH, Haan, Germany) and subsequently ground in a Cyclotec mill (Foss Tecator AB, Höganäs, Sweden) to a 1 mm particle size.

Table 1.
Climatic conditions given as annual values and corresponding data for the maize vegetation period (1st May to 30th September) of mean temperature [°C], mean radiation [J $cm^{-2}$ $d^{-1}$], and precipitation sum [mm] for the experimental years (2001-2003) and the longterm average (1974-2005). Data were kindly provided by the weather station Kiel-Holtenau (54° 22' N, 10° 08'E, 31 m altitude) of the German Weather Service.

| | precipitation [mm] | | temperature [°C] | | radiation [J $cm^{-2}$ $d^{-1}$] | |
|---|---|---|---|---|---|---|
| | annual | veg. period | annual | veg. period | annual | veg. period |
| 2001 | 810.3 | 436.2 | 8.8 | 14.8 | 987.9 | 1658.0 |
| 2002 | 960.9 | 445.3 | 9.7 | 16.4 | 1002.4 | 1651.3 |
| 2003 | 524.1 | 210.1 | 9.6 | 16.7 | 1070.3 | 1731.5 |
| 1974-2005 | 759.1 | 332.4 | 8.7 | 14.8 | 989.1 | 1632.2 |

*2.2 Analysis of cell wall fractions*

The contents of cell wall fractions were estimated using near-infrared reflectance spectroscopy (NIRS). Ground samples were scanned on a NIRSystems 5000 scanning monochromator (FOSS GmbH, Rellingen, Germany), where software (ISI-version) for data collection and manipulation was supplied by Infrasoft International® (ISI, Port Matilda, PA, USA). Calibrations were developed separately for ear and stover. Subsets for calibration and validation were selected from the pooled 2001 and 2002 data, and were extended by 2003 samples. Samples from each calibration and validation subset were analyzed for the content of ash-free neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) according to Goering and van Soest (1970, cited in Naumann and Basler 1976). Ear samples

were pre-treated with heat-stable amylase to ensure starch degradation. Table 2 displays the statistics of the NIRS-calibration and -validation.

The estimated contents of NDF, ADF, and ADL were then used to calculate the hemicellulose and cellulose concentrations of ear and stover according to the following equations: $Hemicellulose = NDF - ADF$ and $Cellulose = ADF - ADL$. Whole plant contents of cell wall fractions were derived from corresponding values of stover and ear and their weight proportions.

Table 2.
Statistics relating to near-infrared reflectance spectroscopy (NIRS) prediction of cell wall fractions, derived by using partial least squares analysis.

| cell wall fraction | n | | mean | | SEC | | $R^2$ | | SEV/SEP | |
|---|---|---|---|---|---|---|---|---|---|---|
| | ear | stover | ear | stover | ear | stover | ear | stover | ear | stover |
| NDF | 51 | 52 | 25.90 | 61.01 | 1.40 | 0.55 | 0.98 | 1.00 | 1.80 | 0.97 |
| ADF | 52 | 52 | 12.07 | 35.83 | 0.69 | 1.08 | 0.98 | 0.97 | 1.05 | 1.27 |
| ADL | 53 | 52 | 4.98 | 6.38 | 0.86 | 0.83 | 0.76 | 0.79 | 1.22 | 1.22 |

n: samples included in the calibration; SEC: standard error of calibration; $R^2$: coefficient of determination; SEV/SEP: standard error of validation

*2.3 Statistical analysis*

A mixed model analysis was calculated using PROC MIXED of SAS 8.2 (SAS Institute Inc., 2001) by considering year, maturity group, variety within maturity group, harvest date, and block as fixed factors and by assuming a heterogeneous, auto-regressive covariance structure for repeated measurements. The resulting model equation for a given cell wall fraction CW was

$$CW_{ijklm} = M + y_i + mat_j + \mathrm{var(mat)}_{jk} + har_l + bl_m + (mat * har)_{jl} + (var(mat) * har)_{jkl} + (y * mat)_{ij} + (y * var(mat))_{ijk} + (y * har)_{il} + (\mathrm{y * mat * har})_{ijl} + (\mathrm{y * var(mat) * har})_{ijkl} + e_{ijklm}$$

where M is the overall mean, $y_i$ is year i, $mat_j$ is maturity group j, $var(mat)_{jk}$: variety k nested in maturity group j, $har_l$ is harvest date l, $bl_m$ is block m, and $e_{ijklm}$ denotes the residual error. Effects were considered significant in all statistical calculations for P-values < 0.05. In case of

significant interactions, linear contrasts were calculated using the SLICE procedure in SAS. Comparison of means was performed by t-test with a Bonferroni-Holm adjustment.

*2.4 Modeling contents of cell wall fractions*

The FOPROQ (FOrage PROduction Quality) model was applied to simulate the contents of NDF, ADF, hemicellulose, and cellulose for whole crop and stover. Model calibration was based on the 2001 to 2003 data. Lignin was not considered for modeling because of insufficient variation of whole crop contents throughout the vegetation period.

FOPROQ consists of two sub-modules, one for DM yield and one for forage quality. The model requires as input daily data on average air temperature [°C], precipitation [mm], potential rates of evapotranspiration [mm], and incident global radiation [MJ $m^{-2}$ $d^{-1}$]. Growth calculations are based on weather data as well as on plant and soil characteristics. Calculation of biomass accumulation starts at sowing or alternatively when a threshold GDD value is reached. Growth is simulated in daily time steps as a function of the current amount of biomass and the relative growth rate, which is a product of the growth potential of the young crop and an age index; the latter describes the impact of crop ageing on growth potential. A growth index, given by the product of the indices of temperature, radiation and plant-available soil water, represents the weather influence on growth. This 'environmental factor' reduces the potential growth rate to an actual rate. Genotypic differences in growth potential are accounted for by the initial amount of biomass, the relative growth rate at the onset of the vegetation period, and the parameters of the age index. For further details on the model algorithms see Herrmann et al. (2005). The sub-module for quality prediction assumes the existence of different levels of quality over the entire growing period, with changes from one level to another occurring gradually. The present model, however, allows only for two such levels. Since then only increasing or decreasing contents of cell wall fractions can be modeled, the data of harvest date 1, which was recorded before silking (see Figs. 4, 5), could not be included in the whole crop simulation, but was considered in stover modeling. The levels and changes in quality depend on input variables reflecting genetics, environment, and management. Environmental factors (as temperature, radiation, and plant-available soil water) are converted into corresponding change rates based on corresponding exponential or negative exponential functions. Parameters $t_c$ / $T_{th}$ (constant and threshold of temperature change function), $r_c$ / $R_{th}$ (constant and threshold of radiation change function)

and $m_c$ (constant in soil moisture change function) determine the slope of the exponential functions for temperature, radiation and soil moisture. Daily environmental change rates are obtained as the product of the rates for temperature, radiation, and plant-available soil water, and are related to forage quality change by an appropriate threshold response function. The curvature of the threshold response functions is controlled by parameters $Q_n$ (minimum content of fiber component at the onset of growth), $Q_x$ (maximum content at maturity), c (determines the inclination of the threshold response curve) and v (determines the inflexion point of the threshold response curve).

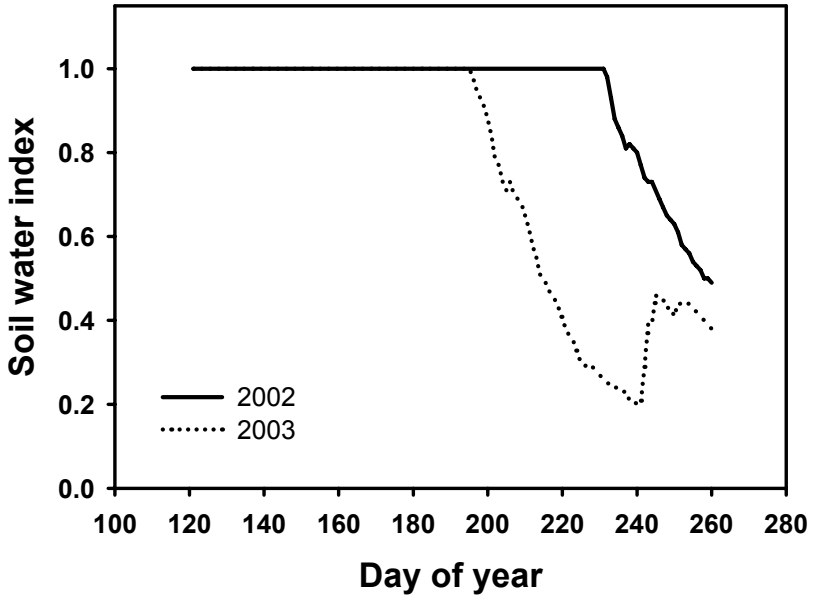


Figure 1.
Calculated soil water index of growing seasons 2002 and 2003, exemplied for cultivar Oldham. The soil water index denotes the ratio of actual to potential plant available soil water.

Environmental conditions may influence the content of cell wall constituents indirectly, if processes like photosynthesis, respiration, morphological development, and ageing are affected (Groot et al., 2003). Apart from these indirect effects, external factors, may affect chemical composition in a direct way. High temperature, for instance, is assumed to accelerate lignification of forage species (Deinum 1984; Wilson et al., 1991). The structure of the FOPROQ model, however, does not allow to differentiate between direct and indirect effects on quality change. When adjusting the model algorithms for cell wall constituents, the indirect impact of external factors on net assimilation, ear fill (Coors et al., 1997), and by extent the dilution of cell wall constituents had to be taken into account. For this reason temperature, radiation,

and water deficit were supposed to accelerate changes of fiber components in the whole crop as well as in the stover (Figs. 1 -3).

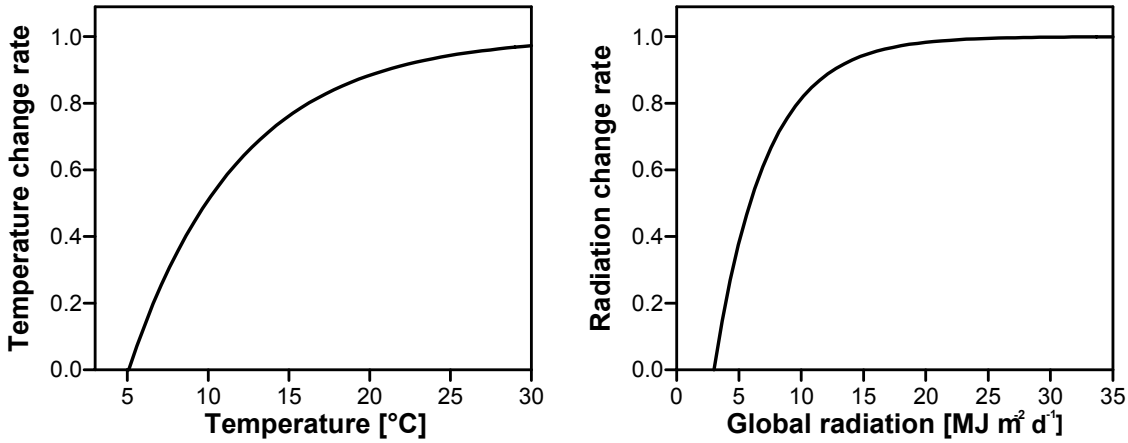


Figure 2.

Temperature change rate and radiation change rate as functions of daily average temperature [°C] and daily sum of global radiation [MJ $m^{-2}$ $d^{-1}$]

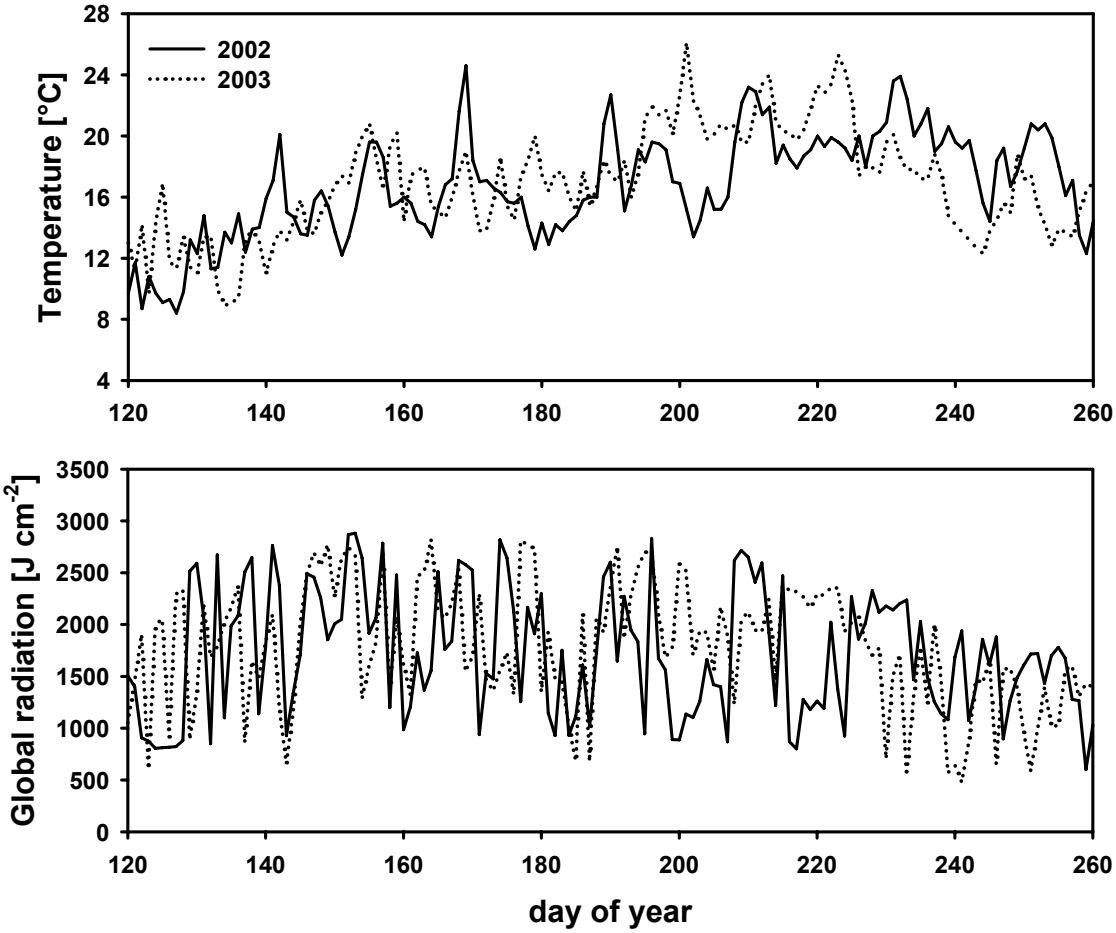


Figure 3.

Daily average temperature [°C] and sum of global radiation [J $cm^{-2}$] of the growing seasons 2002 and 2003.

Model calibration was conducted by an integrated parameter optimization module, which minimizes the deviation between simulated and experimental data in terms of the sum of squared residuals. Model parameters were optimized for each hybrid separately, and model performance was assessed by the root mean square error (RMSE) and the coefficient of determination.

**3. Results**

Samples included in statistical analysis and model calibration covered a wide range of developmental stages as indicated by the DM content of the whole crop (Tab. 3). Silage maturity was achieved at harvest 5 for the early and mid-early maturity group, and at harvest 6 for the mid-late group, if we assume a DM content of 320 g $kg^{-1}$ fresh matter as criteria of silage maturity. The large variation in crop development is reflected by the results of the analysis of variance, where harvest date consistently represented the largest variance component (Tables 4 to 6). Apart from harvest date, environmental conditions had an impact on all measured cell wall fractions of ear, stover and whole crop as indicated by significant main effects of year and of its 2-way interactions with harvest date and maturity (only for whole crop). Genotype had a considerable influence on cell wall fractions as well, as substantiated by significant effects of maturity, variety within maturity, and their interactions with harvest date. The 3-way interaction year x harvest date x maturity was generally significant. Linear contrasts, performed by the SAS slice option in Proc MIXED, however, showed similar effects in each year. Few variety within maturity x harvest date interactions were observed (data not presented), which generally appeared before silage maturity, but showed no consistent trend. The following three sections will therefore focus on the impact of the maturity x harvest date interaction on cell wall fractions of ear, stover, and whole crop. The influence of environmental conditions will be presented in detail in the chapter on modeling results.

Table 3.

Whole plant dry matter content [g $kg^{-1}$ fresh matter] of the tested hybrids provided for the sampling dates as means of the growing periods 2001-2003.

| harvest date | Julian Day | Arsenal (early) | Oldham (early) | Symphony (early) | Probat (mid-early) | Attribut (mid-early) | Fuego (mid-early) | Clarica (mid-late) | Benicia (mid-late) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 187 | 108.05 | 99.40 | 114.77 | 103.89 | 112.20 | 107.06 | 109.44 | 95.65 |
| 2 | 230 | 207.51 | 209.09 | 223.08 | 200.86 | 212.09 | 218.82 | 197.73 | 195.52 |
| 3 | 238 | 231.75 | 223.95 | 252.96 | 220.52 | 233.89 | 246.72 | 216.05 | 221.97 |
| 4 | 246 | 293.68 | 288.68 | 303.53 | 270.21 | 276.20 | 285.92 | 256.06 | 256.21 |
| 5 | 259 | 347.66 | 322.05 | 335.12 | 321.93 | 319.86 | 316.13 | 284.66 | 288.39 |
| 6 | 276 | 393.15 | 407.09 | 391.41 | 388.36 | 385.13 | 369.13 | 333.63 | 327.79 |

Table 4.

F values and level of significances for ear contents of neutral detergent fiber (NDF), acid detergent fiber (ADF), hemicellulose, cellulose, and lignin of eight silage maize hybrids in three maturity groups harvested at six dates in 2001 to 2003.

| ***ear*** | Num DF | F Value | | | | |
|---|---|---|---|---|---|---|
| Effect | | *NDF* | *ADF* | *Hemic.* | *Cell.* | *Lignin* |
| year | 2 | 16.51*** | 59.80*** | 1.53$^{n.s.}$ | 37.74*** | 24.99*** |
| maturity | 2 | 34.82*** | 60.3*** | 15.55*** | 56.46*** | 1.69$^{n.s.}$ |
| var. (mat.) | 5 | 3.92** | 4.38** | 3.38* | 8.48*** | 4.41** |
| har. date | 4 | 438.73*** | 617.36*** | 239.27*** | 484.93*** | 26.8*** |
| block | 1 | 0.27$^{n.s.}$ | 0.35$^{n.s.}$ | 0.01$^{n.s.}$ | 1.86$^{n.s.}$ | 0.21$^{n.s.}$ |
| maturity*har. date | 8 | 5.36*** | 6.85*** | 4.33** | 6.30*** | 0.96$^{n.s.}$ |
| var. (mat.)*har. date | 20 | 2.69** | 2.93** | 2.34** | 2.64** | 1.12$^{n.s.}$ |
| year*maturity | 4 | 1.09$^{n.s.}$ | 1.65$^{n.s.}$ | 0.79$^{n.s.}$ | 1.31$^{n.s.}$ | 0.65$^{n.s.}$ |
| year*var. (mat.) | 10 | 1.17$^{n.s.}$ | 0.96$^{n.s.}$ | 1.39$^{n.s.}$ | 1.38$^{n.s.}$ | 0.93$^{n.s.}$ |
| year*har. date | 8 | 20.41*** | 25.55*** | 13.85*** | 9.88*** | 6.89*** |
| year*har.date*maturity | 16 | 3.60** | 4.54*** | 3.09** | 2.71** | 2.83** |
| year*har.date*var.(mat.) | 40 | 1.03$^{n.s.}$ | 1.27$^{n.s.}$ | 1.02$^{n.s.}$ | 0.85$^{n.s.}$ | 0.78$^{n.s.}$ |

Table 5.

F values and level of significances for stover contents of neutral detergent fiber (NDF), acid detergent fiber (ADF), hemicellulose, cellulose, and lignin of eight silage maize hybrids in three maturity groups harvested at six dates in 2001 to 2003.

| ***stover*** | Num DF | F Value | | | | |
|---|---|---|---|---|---|---|
| Effect | | *NDF* | *ADF* | *Hemic.* | *Cell.* | *Lignin* |
| year | 2 | 54.93*** | 14.93*** | 59.34*** | 55.64*** | 115.49*** |
| maturity | 2 | 63.76*** | 21.71*** | 62.82*** | 39.75*** | 1.60$^{n.s.}$ |
| var. (mat.) | 5 | 7.28*** | 8.44*** | 4.11** | 6.90*** | 5.11** |
| har. date | 5 | 1194.15*** | 1417.64*** | 299.35*** | 1568.70*** | 280.51*** |
| block | 1 | 5.85* | 4.14$^{n.s.}$ | 2.85$^{n.s.}$ | 3.60$^{n.s.}$ | 5.17* |
| maturity*har. date | 10 | 16.13*** | 10.18*** | 7.19*** | 11.19*** | 3.39** |
| var. (mat.)*har. date | 25 | 2.48** | 3.17** | 1.68$^{n.s.}$ | 2.91** | 2.31** |
| year*maturity | 4 | 2.72* | 1.51$^{n.s.}$ | 1.80$^{n.s.}$ | 1.75$^{n.s.}$ | 1.47$^{n.s.}$ |
| year*var. (mat.) | 10 | 2.15* | 1.67$^{n.s.}$ | 1.20$^{n.s.}$ | 1.52$^{n.s.}$ | 0.63$^{n.s.}$ |
| year*har. date | 10 | 48.35*** | 41.03*** | 23.61*** | 60.28*** | 20.11*** |
| year*har.date*maturity | 20 | 3.70** | 4.68*** | 1.76$^{n.s.}$ | 5.55*** | 1.66$^{n.s.}$ |
| year*har.date*var.(mat.) | 50 | 0.91$^{n.s.}$ | 1.30$^{n.s.}$ | 0.93$^{n.s.}$ | 1.50$^{n.s.}$ | 1.31$^{n.s.}$ |

Table 6.
F values and level of significances for whole crop contents of neutral detergent fiber (NDF), acid detergent fiber (ADF), hemicellulose, cellulose, and lignin of eight silage maize hybrids in three maturity groups harvested at six dates in 2001 to 2003.

| ***whole-plant*** | Num DF | F Value | | | | |
|---|---|---|---|---|---|---|
| Effect | | *NDF* | *ADF* | *Hemic.* | *Cell.* | *Lignin* |
| year | 2 | 84.51*** | 93.42*** | 48.17*** | 157.14*** | 65.74*** |
| maturity | 2 | 108.11*** | 166.64*** | 14.79*** | 156.76*** | 3.25* |
| var. (mat.) | 5 | 7.86*** | 7.42*** | 6.36*** | 10.36*** | 2.21$^{n.s.}$ |
| har. date | 5 | 377.15*** | 367.10*** | 178.36*** | 432.77*** | 132.18*** |
| block | 1 | 0.69$^{n.s.}$ | 0.82$^{n.s.}$ | 0.83$^{n.s.}$ | 0.39$^{n.s.}$ | 3.47$^{n.s.}$ |
| maturity*har. date | 10 | 18.92*** | 25.39*** | 3.96** | 32.12*** | 3.46** |
| var. (mat.)*har. date | 25 | 1.90* | 2.67** | 1.36$^{n.s.}$ | 1.96* | 1.54$^{n.s.}$ |
| year*maturity | 4 | 6.74** | 6.86** | 2.78* | 9.65*** | 1.55$^{n.s.}$ |
| year*var. (mat.) | 10 | 1.27$^{n.s.}$ | 1.24$^{n.s.}$ | 1.52$^{n.s.}$ | 1.28$^{n.s.}$ | 0.61$^{n.s.}$ |
| year*har. date | 10 | 30.69*** | 30.07*** | 16.28*** | 34.88*** | 16.74*** |
| year*har.date*maturity | 20 | 3.04** | 4.37*** | 1.60$^{n.s.}$ | 4.12*** | 2.54** |
| year*har.date*var.(mat.) | 50 | 0.96$^{n.s.}$ | 1.35$^{n.s.}$ | 0.75$^{n.s.}$ | 1.08$^{n.s.}$ | 1.20$^{n.s.}$ |

*3.1 Cell wall fractions of the ear*

The contents of NDF, ADF, cellulose, and hemicellulose showed a continuous decline over the vegetation period (Figs. 4, 5) due to the accumulation of starch. Contents of NDF, ADF, and cellulose almost always differed significantly among the maturity groups at dates 2 to 5, except, for instance, between the mid-early and mid-late groups at date 2. Largest differences among the maturity groups were detected at harvest date 3, where the mid-late group exceeded the early group by 79.0 g NDF kg$^{-1}$ DM, 45.0 g ADF kg$^{-1}$ DM, 36.3 g cellulose kg$^{-1}$ DM, and 34.1 g hemicellulose kg$^{-1}$ DM. With increasing maturity, differences diminished, so that at harvest date 6, significances were observed only for the comparison of early vs. mid-late (ADF) and of mid-early vs. mid-late (cellulose). In contrast to NDF, ADF, and cellulose, the content of hemicellulose revealed significant differences mainly up to harvest date 3. Lignin content showed a small decline over vegetation period. Yet, the interaction variety within maturity x harvest date was not significant.

*3.2 Cell wall fractions of the stover*

In stover, advancing maturation resulted in substantial increase of NDF, ADF, hemicellulose, and cellulose, amounting to 239.1 g NDF kg$^{-1}$ DM, 168.6 g ADF kg$^{-1}$ DM, 70.5 g hemicellu-

lose kg$^{-1}$ DM, and 142.1 g cellulose kg$^{-1}$ DM for the early maturity group for instance (Figs. 4, 5). There was fewer significance among maturity groups at harvest dates 1 and 2, i.e. before and shortly after silking. Differentiation among maturity groups became more evident at harvest dates 3 to 5, except for ADF, where the mid-early and mid-late group differed significantly only at date 5. Largest differences among maturity groups were recorded at harvest date 5 (amounting to 58.1 g NDF kg$^{-1}$ DM, 31.0 g ADF kg$^{-1}$ DM, 27.6 g hemicellulose kg$^{-1}$ DM, and 27.9 g cellulose kg$^{-1}$ DM between the early and mid-late group), but decreased again afterwards. At harvest date 6, ADF content did not show any significant difference among maturity groups, while for NDF and hemicellulose early vs. mid-late and mid-early vs. mid-late differed significantly, and for cellulose mid-early vs. mid-late was non-significant. The lignin content increased from 44.3 g kg$^{-1}$ DM to 75.5 g kg$^{-1}$ DM (on average of the maturity groups) without any obvious trend concerning the ranking of the groups.

*3.3 Cell wall fractions of the whole crop*

The whole crop content of fiber fractions reflects the growing ear/stover ratio (Figs. 4, 5). Between harvest date 1 and 2, i.e. around silking, contents showed an increase for the mid-late group, while for the early and mid-early group the increase was less pronounced (NDF, ADF) or rather non-existent (hemicellulose). After the onset of grain filling, remobilization of assimilates and starch accumulation resulted in a considerable decline, varying between 103.9-117.6 g NDF kg$^{-1}$ DM, 61.5-69.5 g ADF kg$^{-1}$ DM, 40.0-47.2 g hemicellulose kg$^{-1}$ DM, and 55.8-67.1 g cellulose kg$^{-1}$DM.

In agreement to the ear, largest differences among maturity groups were detected at harvest date 3, with 58.1 g NDF, 43.0 g ADF, 37.5 g cellulose, and 15.2 g hemicellulose kg$^{-1}$ DM between the early and mid-late group. While for NDF, ADF, and cellulose the groups differed significantly at all harvest dates, except for early vs. mid-early at date 2 and early vs. mid-early at date 6 (cellulose only), hemicellulose showed fewer significances at early growth stages and maturity groups did not differ at harvest date 6. Lignin content increased from 1$^{st}$ to 2$^{nd}$ harvest date, and then remained relatively stable. There were only few significances, which did not indicate any clear trend.

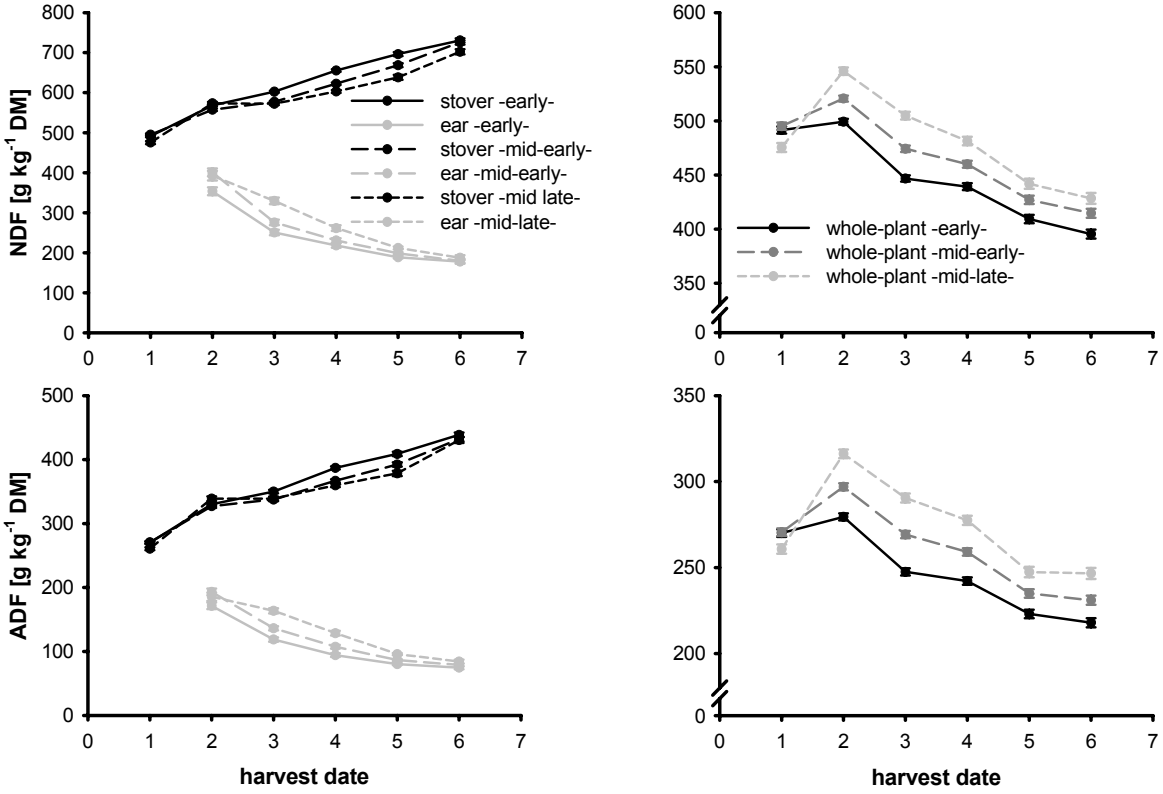


Figure 4.

Content of neutral detergent fiber (NDF) and acid detergent fiber (ADF) of ear, stover and whole-plant of early, mid-early and mid-late maturity group, mean of three experimental years (2001-03). Bars representing standard errors.

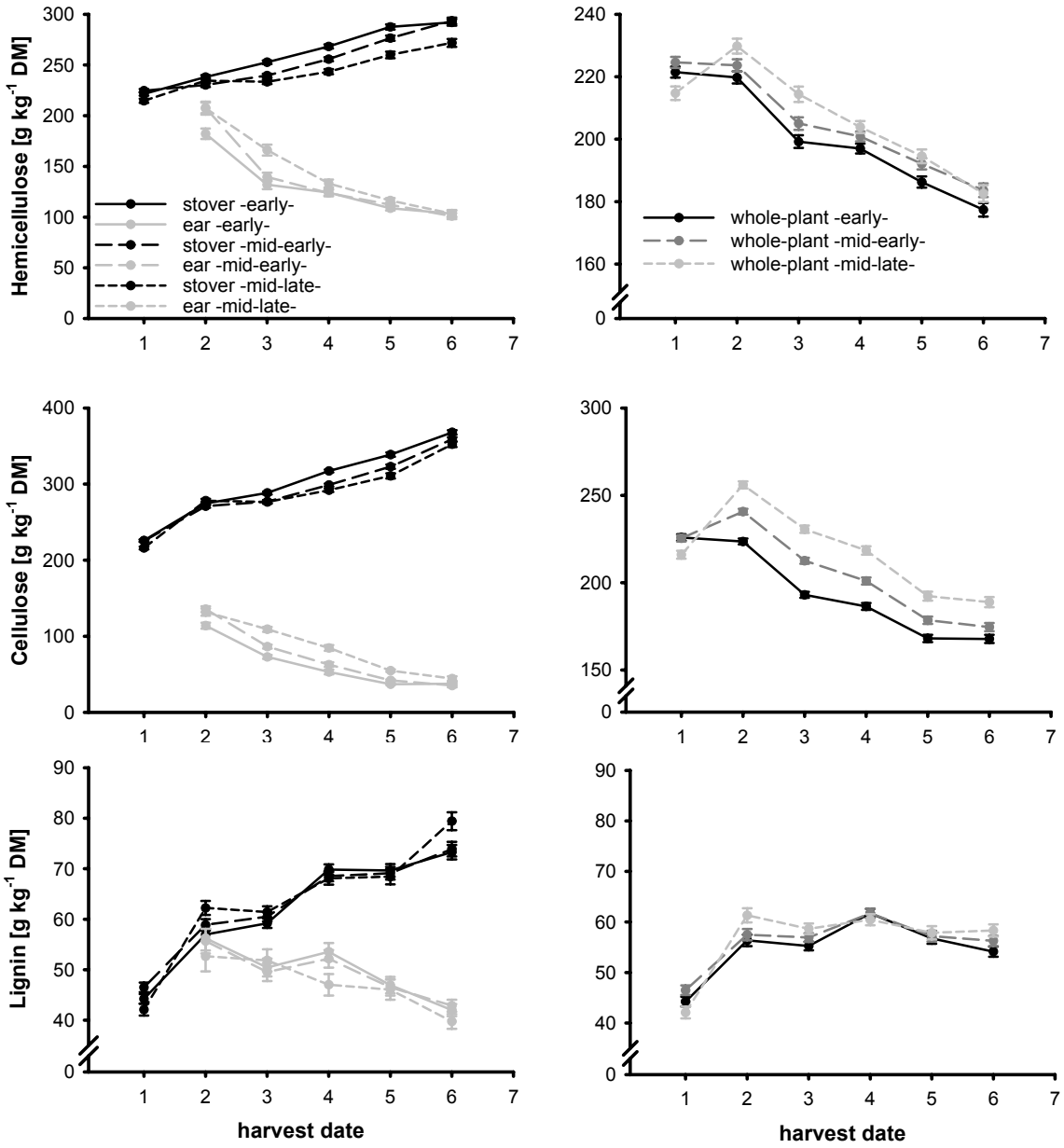


Figure 5.

Content of cellulose, hemicellulose and lignin of ear, stover and whole plant of the early, mid-early and mid-late maturity group, mean of three experimental years (2001-03). Bars representing standard errors.

*3.4 Modeling of fiber fractions*

The contents of fiber fractions were strongly influenced by the year, as shown before. By applying the FOPROQ model, the less meaningful factor year was replaced by weather variables in order to quantify separately the effect of temperature, precipitation, and radiation on the content of fiber components. Model calibration was performed separately for each hybrid. For the sake of clearness, however, we restrict the presentation of results to hybrid Oldham, which belongs to the early maturity group and is characterized by synchronous maturation of ear and stover, and to Fuego, a mid-early variety with stay green behavior. Other hybrids reacted in a similar manner and calibration resulted in comparable goodness of model fit. We furthermore restricted modeling to stover and whole crop, since the ear contributes less to the fiber production of the whole crop.

*3.4.1 NDF, ADF, hemicellulose, and cellulose content of the stover*

Fig. 6 displays the modeled and observed stover contents of NDF, ADF, hemicellulose, and cellulose for the selected varieties. In the growing period of 2003, environmental conditions led to an earlier and more intense increase of NDF, ADF, hemicellulose, and cellulose compared to 2001 and 2002 for both varieties. Although the 2002 growing season had much less severe soil water deficit compared to 2003, as indicated by the water index WI (Fig. 1), model optimization resulted in a negligible contribution of soil water availability. Thus, mainly temperature and radiation determined the intensity of quality change (Fig. 2). This is somewhat surprising, since mean temperature and radiation during vegetation period was only slightly higher in 2003 compared to 2002. However, temperature and radiation differed in certain periods (Fig. 3). The impact of temperature and radiation on the daily quality change rates, i.e. parameters $t_c$, $T_{th}$, $R_c$, $R_{th}$ (Tab. 7) revealed no differences among cell wall constituents and hybrids. Values for $Q_x$, the maximum content of a given fiber component at maturity have no physiological meaning, but were caused by the experimental design since in some years, the maize crop was harvested before physiological maturity.

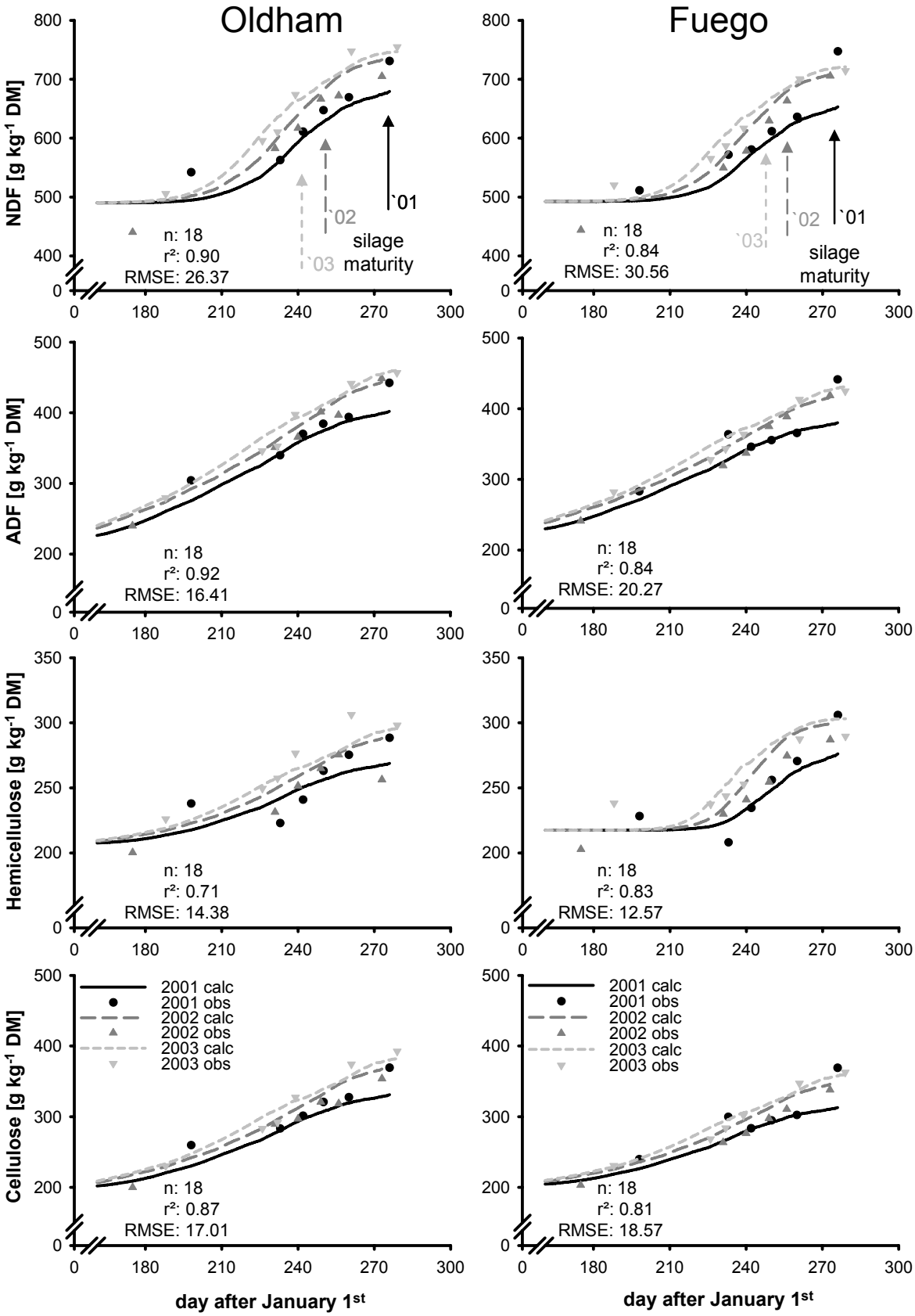


Figure 6.

Observed (symbols) and calculated (lines) values of neutral detergent fiber (NDF), acid detergent fiber (ADF), hemicellulose and cellulose in the stover of the varieties Oldham and Fuego (2001-2003). Arrows indicate silage maturity in corresponding years (~320 g DM $kg^{-1}$).

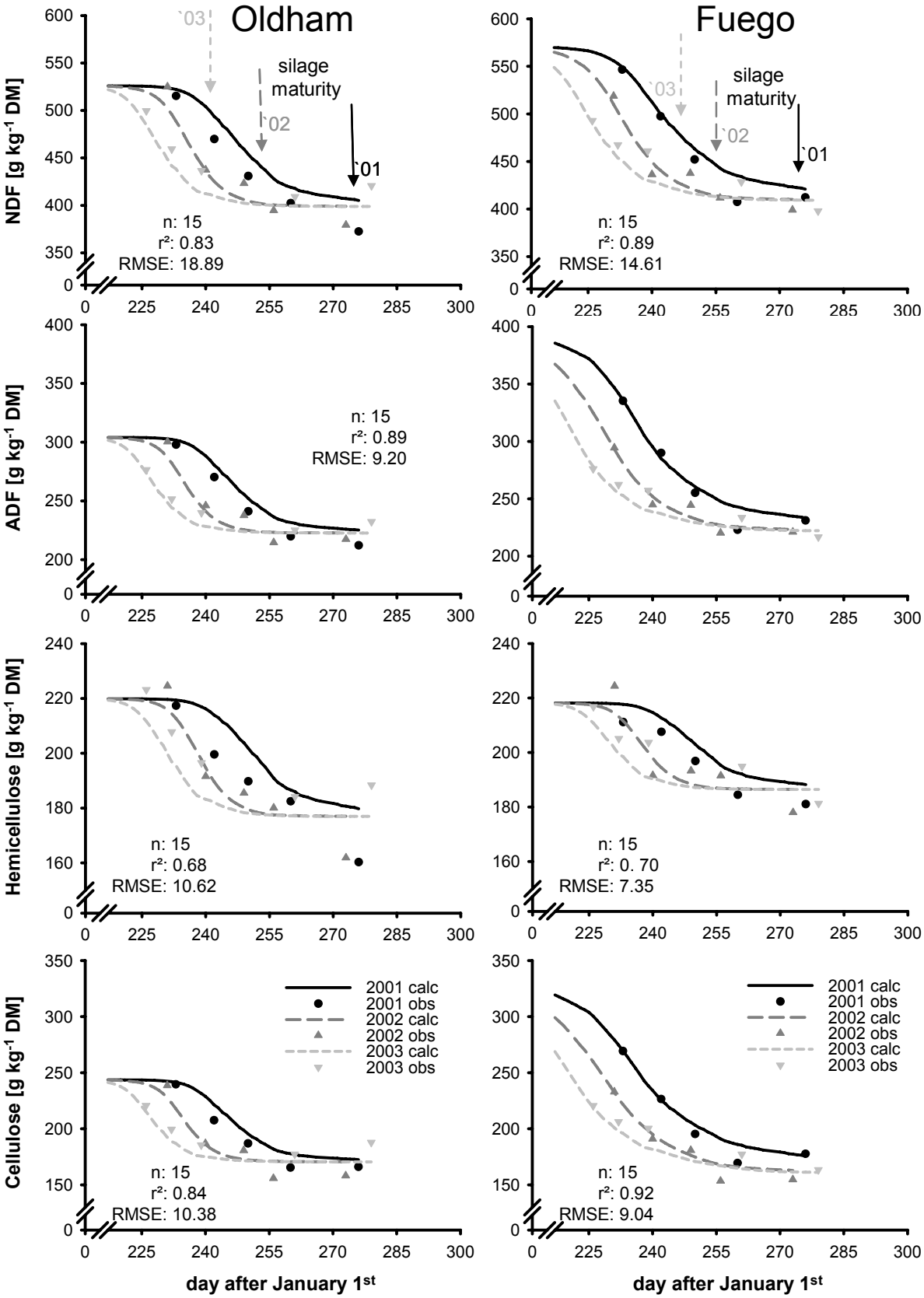


Figure 7.

Observed (symbols) and calculated (lines) data of neutral detergent fiber (NDF), acid detergent fiber (ADF), hemicellulose and cellulose in the whole-plant of the varieties Oldham and Fuego (2001-2003). Arrows indicate silage maturity in corresponding years (~320 g DM $kg^{-1}$).

Table 7.

Results of parameter estimation of the FOPROQ quality submodel.

| | NDF | | ADF | | Hemicellulose | | Cellulose | |
|---|---|---|---|---|---|---|---|---|
| *Oldham* | whole plant | stover | whole plant | stover | whole plant | stover | whole plant | stover |
| $t_c$ | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 |
| $T_{th}$ | 6.00 | 6.00 | 6.00 | 6.00 | 6.00 | 6.00 | 6.00 | 6.00 |
| $r_c$ | 0.24 | 0.24 | 0.24 | 0.24 | 0.24 | 0.24 | 0.24 | 0.24 |
| $R_{th}$ | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 |
| $m_c$ | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 |
| $Q_n$ | 39.89 | 49.01 | 22.27 | 19.99 | 17.70 | 20.61 | 17.06 | 19.36 |
| $Q_x$ | 52.60 | 79.34 | 30.41 | 2588.92 | 21.99 | 47.74 | 24.37 | 144.99 |
| $v$ | 91.34 | 94.58 | 90.52 | 2266.45 | 93.17 | 151.22 | 90.30 | 261.20 |
| $c$ | 28.16 | 7.34 | 30.69 | 1.56 | 31.49 | 3.00 | 31.18 | 2.22 |

| | NDF | | ADF | | Hemicellulose | | Cellulose | |
|---|---|---|---|---|---|---|---|---|
| *Fuego* | whole plant | stover | whole plant | stover | whole plant | stover | whole plant | stover |
| $t_c$ | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 |
| $T_{th}$ | 6.00 | 6.00 | 6.00 | 6.00 | 6.00 | 6.00 | 6.00 | 6.00 |
| $r_c$ | 0.24 | 0.24 | 0.24 | 0.24 | 0.24 | 0.24 | 0.24 | 0.24 |
| $R_{th}$ | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 |
| $m_c$ | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 |
| $Q_n$ | 40.87 | 49.25 | 22.09 | 20.91 | 18.64 | 21.75 | 15.88 | 19.89 |
| $Q_x$ | 57.09 | 75.51 | 39.39 | 2557.69 | 21.82 | 30.86 | 33.18 | 141.76 |
| $v$ | 89.04 | 96.26 | 84.74 | 2275.56 | 92.47 | 96.92 | 84.63 | 265.69 |
| $c$ | 19.29 | 8.85 | 14.36 | 1.61 | 30.84 | 13.26 | 12.37 | 2.36 |

with $t_c$ : constant of temperature change function, $T_{th}$ : temperature threshold in temperature change function, $r_c$ : constant of radiation change function, $R_{th}$ : radiation threshold in radiation change function, $m_c$ : constant in soil moisture change function, $Q_n$ : minimum content of fiber component at the onset of growth, $Q_x$ : maximum content at maturity, $c$ : determines the inclination of the threshold response curve, and $v$ : determines the inflexion point of the threshold response curve.

Dry matter contents suitable for ensiling (~320 g DM $kg^{-1}$) were achieved 20 days earlier in 2002 compared to 2001 and 35 days earlier in 2003, whereas for Fuego harvest dates around 320 g DM $kg^{-1}$ failed in 2003. Calculated fiber values at silage maturity (~320 g DM $kg^{-1}$) differed by 42.5 g NDF $kg^{-1}$ DM, 26.5 g ADF $kg^{-1}$ DM, 12.3 g hemicellulose $kg^{-1}$ DM, and 23.1 g cellulose $kg^{-1}$ DM between 2003 and 2002 for Oldham. A similar range was detected for Fuego. Variations in NDF and hemicellulose content between varieties were largest in

2003, where NDF content, for instance, was 24.7-27.1 g $kg^{-1}$ DM higher for Oldham compared to Fuego. The comparison of calculated and observed data in Fig. 6 demonstrates that fiber fractions were reasonably well estimated for NDF, ADF, and cellulose in both cultivars, explaining 81-92% of variation. Prediction errors (RMSE) ranged below 5.27% of the contents at silage maturity. Some discrepancies became apparent for the last harvest date of 2001, where the model systematically underestimated the observed values. For hemicellulose model fit was less satisfactory, especially the 2001 data were not well matched.

*3.4.2 NDF, ADF, hemicellulose, and cellulose content of the whole crop*

The calculated and measured whole-crop contents of fiber fractions are presented in Fig. 7. In agreement to the stover results, 2003 was characterized by a faster quality change, especially for NDF, hemicellulose, and cellulose, where the decrease started earlier. In contrast to the stover, the differentiation among years seems more pronounced for NDF, ADF, and cellulose during early maturation. With advancing maturity, however, the contents converged. Consequently, the concentrations of the whole crop at silage maturity differed less among years. Calculated cell wall constituents of Oldham in 2003, for instance, exceeded the 2002 values by 13.1 g NDF, 5.7 g ADF, 6.3 g hemicellulose, and 4.6 g cellulose. The same trend was observed for Fuego. In agreement to the stover, temperature and radiation were the main drivers of quality change, while soil water availability had essentially no impact. Parameters for temperature and radiation change rate curves correspond to those estimated for the stover (Tab. 7). Agreement between simulated and measured concentrations was satisfactory for NDF, ADF, and cellulose, with $R^2$ values above 0.83 and RMSE values around 5% of the final quality values. Hemicellulose calculations showed a somewhat higher variation, especially for Oldham, where 2001 values were overestimated and 2003 data were slightly underestimated.

## 4. Discussion

*4.1 Genotypic variation of fiber components*

Hybrids showed a typical pattern of changes of fiber components in ear, stover, and whole crop over time (Figs. 4, 5). Decreasing contents in the ear and the whole-plant (except for lignin) and increasing stover concentrations with advancing maturation generally agree with

previous investigations (Phipps and Weller, 1979; Russell, 1986; Cone and Engels, 1993; Irlbeck et al., 1993; Tolera et al., 1998; Darby and Lauer, 2002). Likewise, the range of fiber contents recorded at silage maturity closely matches those obtained by other studies (Cox et al., 1994; Verbic et al., 1995; Darby and Lauer, 2002). Opposite to Bal et al. (1997) and Wiersma et al. (1993), the underlying study does not support increasing whole crop fiber contents after physical maturity.

Varietal differences in the content of fiber fractions were marginal, i.e. only a few significances in early stages of development were observed, which might be of interest for anaerobic digestion of maize for the purpose of biogas production. The optimum harvest time of maize grown for biogas production is discussed intensively at present in Germany. Up to now, there is no consensus on it, but recent research suggests that an earlier harvest time compared to silage maize production might be advantageous (Amon et al., 2004). At silage maturity, in contrast, we did not find any impact of variety on fiber components. Even the stay green behavior (mid-early cultivar Fuego and mid-late Benicia) did not show any effect, although results by Ettle and Schwarz (2003) give reason to suppose that a later onset of senescence could result in a slower increase of fiber components. It may be argued that the number of hybrids tested in our study was not sufficient to draw a general conclusion on genotypic variability of fiber content. The hybrids, however, were specifically selected according to their maturation behavior in order to represent the spectra of German silage maize varieties. In addition, our findings are in agreement with Darby and Lauer (2002), who found a lack of hybrid differences and interactions throughout their experiment, suggesting that hybrid quality varied similarly across harvest dates. Likewise, Crasta et al. (1997) did not find any hybrid impact on cell wall constituents of whole crop and stover. Genotype x environment interactions reported in other studies (Deinum, 1988; Kang and Gorman, 1989; Giauffret et al., 2000; Epinat-Le Signor et al., 2001), however, do not allow to reason that there is a general lack of genetic variability, since hybrid performance may change with environmental conditions. Additional support for some genotypic variability comes from the study of Barrière et al. (1991), who documented a range of 407-496 g NDF $kg^{-1}$ DM, 195-249 g ADF $kg^{-1}$ DM, and 171-229 g cellulose $kg^{-1}$ DM. Since, however, the dry matter content of samples varied between 295 and 388 g $kg^{-1}$ fresh matter, we may assume that part of the variation was caused by differences in developmental stage, but not completely by genotypic variation.

Likewise, differences in developmental stage are responsible for most of the maturity x harvest time interactions detected in our study. The field experiment was designed to harvest all hybrids at the same date. Consequently, the hybrids belonging to the early maturity group were in a more advanced maturity stage, characterized by higher ear-to-stover ratios, and higher starch contents, and hence resulted in systematically lower or higher fiber contents, depending on plant part and growth phase (Figs. 4, 5). The varying ear-to-stover ratio may also explain why we observed only few significances among maturity groups at late growth stages in ear and stover, whereas for the whole crop differences were more pronounced. For this reason comparisons to determine the genotypic variability were useful only within maturity groups. Comparisons between maturity groups allow to assess the switch for instance from an earlier to a later maturity group, i.e. the impact of the time period required from planting to silage maturity.

The alternative approach, i.e. harvesting at similar developmental stages, would have implicated different environmental conditions during growth, and thus would not have allowed to quantify the genotypic impact on the content of cell wall constituents.

*4.2 Effects of environmental variation on fiber components*

Contents of cell wall constituents showed a substantial variation due to environmental conditions, as indicated by significant effects of the year and corresponding interactions (Tables 4, 5, 6). Results of model optimization revealed that the intensity of change in fiber components was strongly associated with temperature and radiation. These findings generally agree with the work of Cox et al. (1994), who found high temperature to reduce whole crop fiber concentration. In contrast, Crasta et al. (1997) reported only a small, but significant contribution of temperature to NDF, ADF, and lignin variability at silage maturity. Soil water availability did not contribute significantly to variability of fiber components in our study, whereas Crasta et al. (1997) documented water availability to be closer related to content of fiber components than temperature. Overall, however, they were able to explain only 48 to 55% of variability at silage maturity. Presumably, these controversial results may due to differences in the level of water deficiency. According to Grant et al. (1989), water use of maize remains unaffected by water availability unless the soil moisture index, i.e. the ratio of the actual to the maximum plant available soil water, falls below 0.2 to 0.3. In the growing season 2003 of our study,

these values were not met until 14 days after silking (day 208), see Fig. 1, when the most sensitive period with respect to kernel set (Grant et al., 1989) was nearly over. In summary, the model was able to reflect the impact of environmental conditions on the changes of cell wall constituents during the vegetation period satisfactorily. For unknown reasons, the correspondence between measured and simulated values was less close for hemicellulose.

In order to obtain a more comprehensive assessment of the environmentally caused variation of fiber components, we applied the calibrated model to perform a complementary long-term simulation study, based on 30 years of weather records (1976-2005). The simulation study was conducted using the module FOSIM, which allows to run FOPROQ with a prespecified harvest strategy, controlling the harvest time by setting target values concerning harvest date, DM yield, and/or forage quality. As soon as one of the target values was reached, the simulation run was terminated. We set the harvest time for both varieties in the study at a whole crop DM content of 320 g DM $kg^{-1}$ fresh weight. For unfavorable climatic conditions with delayed maturity we set $10^{th}$ October as the latest possible harvest date if silage maturity was not reached until then. Figure 8 displays the results of the simulation study. The location of the distributions was characterized by their mean and median, because, apart from NDF, all distributions were right skewed. The contents of cell wall constituents showed a noticeable weather related variation, as indicated by coefficients of variation between 3.6 and 8.9%. Hemicellulose contents, however, seem to be somewhat less affected by environmental conditions compared to the other fiber components, especially for Fuego. The striking outliers in the upper (fiber components) or lower range (DM content) of the distributions, consistently can be traced back to the year 1987, which was characterized by extremely low temperature (12.9 °C vs. 14.8 °C long term average) and radiation (1299 vs. 1632 J $cm^{-2}$) during the vegetation period. It seems likely that the observed variation is mainly due to the impact of weather conditions on the development of the crop, i.e. by indirect effects on fiber components, because the weather related variability of DM content was considerably larger. The silage maturity target (320 g DM $kg^{-1}$ fresh weight) was missed in 67% for both cultivars, and in 43% of all years the DM content was below 29%

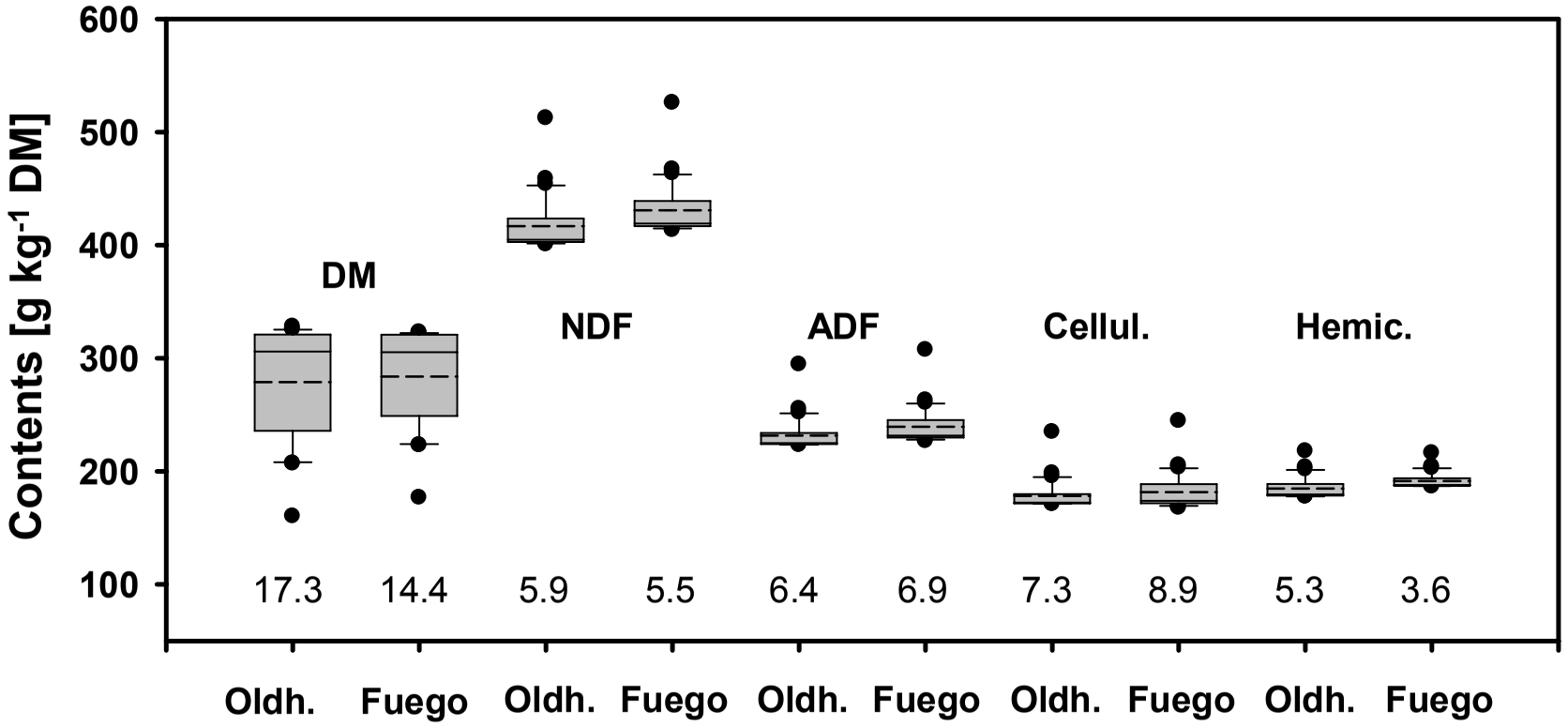


Figure 8.

Results of the 30-year simulation study of DM content [g DM $kg^{-1}$ fresh weight], and of concentrations of NDF, ADF, cellulose, and hemicellulose [g $kg^{-1}$ DM] at harvest for hybrids Oldham and Fuego, given as box plots with 10, 25, 50, 75, and 90% quantiles, outliers (•), and mean (----). The figures below the box plots provide the coefficients of variation [%].

which is regarded as a lower limit for successful ensiling. Comparable ratios have been obtained in an earlier study (Herrmann et al., 2005). Hybrids Oldham and Fuego showed rather similar distributions, with slightly higher mean/median values for Fuego, although they represent different maturity groups. In summary, the results of the simulation study document a strong weather related variation for the DM content and a moderate influence on the content of cell wall constituents. Compared to environmental conditions, the impact of variety seems insignificant. Méchin et al. (2000), in contrast, detected a substantial variation in stem NDF content; kernel starch accumulation, however, may dilute these differences with respect to whole crop fiber content.

*4.3 Model approach*

The FOPROQ model, originally developed for forage grasses, could be applied to maize whole crop and stover fiber components without any modifications of model algorithms. Promising results of model calibration corroborate the model´s general suitability. The underlying model concept, i.e. the dynamic simulation of daily changes in forage quality traits driven by weather factors, seems to have several advantages over less complex model approaches. Thermal indices, for instance, are widely applied to predict harvest time or forage quality traits (Dwyer et al. 1999; Easson and Fearnehough 2003; Bloc et al. 1983; AGPM 2000; Dardenne et al. 1993; Van Soest and Hall 1998). However, additional climatological data may be required to reliably predict forage quality (Hill et al., 1995).

Despite the promising results, FOPROQ performance might be improved by introducing some model refinements. The study of Struik et al. (1985), for instance, supposes that temperature influence on cell wall content is most significant during the period from the 8-leaf stage to grain set. Consequently, distinguishing the crop sensitivity to external factors in different developmental stages, might enhance model power. This partitioning would allow to determine response functions separately for each developmental period in order to quantify the impact of environmental conditions more accurately. Such approach is supported by Stewart et al. (1998), who found substantial differences in the phenological temperature response between the vegetative and reproductive growth stages. The increase of model complexity, however, should be well considered. A higher degree of complexity does not necessarily result in improved prognosis accuracy, because overparameterization may increase prognostic uncertainty (Jansen, 1998).

## 5. Conclusions

The results from the field experiment revealed only little genotypic variability in concentration of cell wall constituents of the whole crop as well as of the stover. Few significant differences between hybrids within a given maturity group were observed. However, they occurred only in early stages of development, which are of no importance with respect to ruminant nutrition, but may possibly be of interest for methane production in biogas plants. In contrast, environmentally caused variation of fiber contents was more pronounced. The application of the FOPROQ model allowed to quantify the impact of weather factors on the day-to-day changes of NDF, ADF, cellulose, and hemicellulose. Surprisingly, temperature and radiation turned out to be the major determinants of content of cell wall components, while soil water availability was of minor importance. Potentially, drought stress conditions in the underlying field study were not severe enough or occurred too late in the growing period. Measured data were predicted with acceptable accuracy, but model performance could be further enhanced by distinguishing crop response to environmental stress factors depending on developmental stage. An additional long-term simulation study allowed to quantify the environmental variability of whole crop fiber components comprehensively, showing a considerable variation, which was mainly due to environmental impacts on developmental stage, as indicated by the impact on dry matter content.

Before introducing the model as a prognosis tool into practical agriculture, the data base underlying model calibration should be extended, in order to improve the reliability of model output with respect to other regions differing in weather conditions. Furthermore, the model requires validation with an independent data set.

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# Kapitel 4

# Characterization of silage maize varieties with content of water soluble carbohydrates during the vegetation period and weather-based modeling

Sandra Kruse, Antje Herrmann, Alois Kornher, Friedhelm Taube

**Abstract**

The concentration of water soluble carbohydrates (WSC) is a main determinant of the ensiling potential of forages and the aerobic stability of silages. There is, however, only little information available about the contribution of genotype and environmental conditions on the WSC content of silage maize. The aims of the present study therefore were to assess the impact of genotype on the seasonal changes of WSC concentration, to adapt and calibrate the FONSCH model, originally developed for forage grasses, for silage maize, and finally to conduct a long-term simulation study for quantifying the weather-related variability of WSC content. A three-year field experiment (2001-2003) was conducted in Northern Germany to evaluate differences in WSC content among commercial silage maize varieties across different growing conditions, and to serve as calibration data base for modeling. Eight varieties covering three maturity groups (early, mid-early, mid-late) and different maturation types (dry-down, normal, stay-green) were investigated. At six dates throughout the vegetation period the plants were harvested, separated into ear and stover, and freeze-dried for subsequent determination of WSC. The WSC concentration of ear, stover, and the whole crop was significantly influenced by genotype and environmental conditions. Genotypic differences were most pronounced in the early grain filling period, amounting in the whole crop to a maximum of 71.43 g WSC $kg^{-1}$ DM among maturity groups and 55.05 g WSC $kg^{-1}$ DM within groups. At silage maturity, a significant effect of variety was found in the mid-early group only, accounting for a difference of 44.83 g WSC $kg^{-1}$ DM. Model calibration showed good agreement between observed and calculated WSC contents, with RMSE below 26.0 g WSC $kg^{-1}$ DM and $R^2$ above 0.84. Parameter estimates revealed a substantial influence of temperature and solar radiation on WSC content. Plant available soil water had essentially no impact, although the growing season 2003 was characterized by severe water shortage after silking. Successful model calibration allowed to perform a 30-year simulation study for Kiel, representing marginal regions of maize production in Northern Germany. Variation among years above 92.0 g WSC $kg^{-1}$ DM indicates a considerably larger impact of environmental conditions compared to genotype.

**Keywords**

silage maize, variety, environmental conditions, water soluble carbohydrates, modeling

**Abbreviations**

ADF: acid detergent fiber; a: constant determining the curvature of the function for LAI calculation; *ach, bch, cch* : constants determining the curvature of function, which relates the accumulated daily environmental change rates to the WSC content; BBCH: Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie; DM: dry matter; ISI: Infrasoft International; LAI: leaf area index; $m_c$ : constant in soil moisture change function; MPLS: Modified Partial Least-Squares method; N: nitrogen; NDF: neutral detergent fiber; NIRS: Near Infrared Reflectance Spectroscopy; r: relative growth rate at onset of growth; $R^2$: coefficient of determination; $r_c$: constant of radiation change function; $R_{th}$: radiation threshold in radiation change function; RMSE: root mean squared error; $t_c$ : constant of temperature change function; $T_{th}$: temperature threshold in temperature change function; WSC: water soluble carbohydrates $W_0$: aboveground biomass at onset of growth

## 1. Introduction

Over the last decades, maize has become a key to high production on livestock farms, mainly proven on its ability to produce high quality silage, and recently gained importance as substrate for biogas production in Germany. Fermentation, however, may be limited due to low contents of water soluble carbohydrates (WSC) under unfavorable environmental conditions, while high contents in silage may restrict aerobic stability during the feed-out phase (Pahlow et al., 2003). With advancing maturation of the maize crop, carbohydrate composition undergoes systematic changes (McAllen and Phipps, 1977; Phipps and Weller, 1979). During vegetative development WSC are accumulated in leaves and stems, optimal growing conditions provided (Phipps et al., 1984). After silking, the developing kernels become the primary sink for photosynthate produced by the plant (Tollenaar, 1977; Hawker et al., 1991; Schussler and Westgate, 1994). Starch accumulation typically is paralleled by a decline in WSC content under temperate climatic conditions (McAllen and Phipps, 1977; Phipps and Weller, 1979; Russell, 1986; Oldenburg and Laws, 1993).

Consistently, environmental conditions are regarded as a key determinant of maize development (Struik, 1983; Wilson et al., 1995; Ben Haj Salah et al. 1996; Bos et al., 2000; Andresen et al., 2001; Epinat-Le Signor et al., 2001), influencing photosynthetic acitivity directly and indirectly (Teeri et al., 1977; Miedema, 1982; Long, 1983; Fryer et al., 1995, 1998; Ying et al., 2002). Adverse weather conditions, e.g. soil water deficiency as well as low

temperature and solar radiation, may modify the source-to-sink ratios, the assimilate translocation from stover to ear, and by extent the carbohydrate composition of the crop (Struik, 1983; Phipps et al., 1984; Lu et al., 1996; Coors et al., 1997). This applies especially if stress conditions occur during sensitive stages of maize development. For water-soluble carbohydrates, several studies detected a significant influence of the experimental year (Cummins, 1970; Russell, 1986; Andrieu et al., 1993). Apart from environmental conditions, maize genotype can affect the development and the metabolic activity of the plant. Significant differences in whole crop WSC content among hybrids were reported by Wilkinson and Phipps and Weller (1979), whereas Andrieu et al. (1993) and Irlbeck et al. (1993) found little or no impact of genotype on total non-structural carbohydrates. There is little information about the genotypic variation of WSC content in recent hybrids grown in Germany. Likewise, there is a lack of knowledge about the quantitative relationship between WSC content and weather factors (temperature, precipitation, radiation).

This gap might be filled by the development of an appropriate model for analyzing the crop´ s response to environmental conditions. Existing maize models, which are based on radiation use efficiency as a key determinant of growth, such as CERES (Jones and Kiniry, 1986) and CropSyst (Stöckle et al., 1994), typically do not include algorithms for calculating carbohydrate reserves. Some of the assimilate driven models with explicit functions for photosynthesis and respiration consider the accumulation of reserve carbohydrates (Spitters et al., 1989), while others do not (Yang et al., 2004). Complex mechanistic models are usually assumed to have a relatively high model performance compared to simpler approaches, their practical application, however, may be limited due to the larger need for input data. The weather-based, dynamic FONSCH model (Wulfes et al., 1999), developed to simulate the content of WSC in forage grasses, constitutes a useful compromise between empirical and complex, dynamic approaches.

The purposes of the study presented here were (i) to assess the impact of genotype on seasonal changes of WSC content, (ii) to investigate, if the FONSCH model is applicable to forage maize, and finally (iii) to quantify, by means of a 30-year simulation study, the influence of environmental conditions on WSC content for regions of Northern Germany.

## 2. Materials and Methods

### *2.1 Field experiment*

The study was based on data collected in a 3-year (2001-03) field experiment conducted at the experimental farm 'Hohenschulen' (53°18'N, 9°58'E, 32 m altitude) of the Faculty of Agricultural and Nutritional Science at the University of Kiel in Northern Germany. The soil type at 'Hohenschulen' can be classified as pseudogleyic sandy loam. The prevailing climate at the experimental site is humid-temperate, with an average annual rainfall of 733 mm and daily mean temperature of 8.6°C (1974-2005). Daily mean temperature, daily mean radiation and rainfall in the considered vegetation periods (1$^{st}$ May to 30$^{th}$ September) are provided in Table 1.

A one-factorial block design with two replicates (plot size 90 m²) was used for the field trial, where eight varieties (Arsenal, Oldham, Symphony, Probat, Attribut, Fuego, Clarica, Benicia), covering a wide maturity range relevant for Germany (early to mid-late) and different maturation types (dry-down, normal and stay-green; high to low harvest index), were investigated. Maize was sown in early May (2$^{nd}$ in 2001, 10$^{th}$ in 2002 and 5$^{th}$ in 2003) in rows 0.75 m apart, with a final plant density of 9-10 plants m$^{-2}$. Nitrogen fertilization (150 kg N ha$^{-1}$) was split into 3 applications: before planting, first-leaf-stage and 6-8-leaf stage. Phosphorous ($P_2O_5$), potassium ($K_2O$) and magnesium (MgO) were applied at 40, 250, and 30 kg ha$^{-1}$, respectively. Plant protection was conducted according to the codes of 'Good Agricultural Practice in Plant Protection and Fertilization'. After emergence, crop phenology and occurrence of key growth stages e.g. tasseling and silking was recorded. Sampling dates of all varieties were chosen in order to be in line with developmental stages of the variety Probat (mid-early maturity group, synchronous maturation of ear and stover), scheduled to BBCH 32 and an ear DM content of 20, 30, 40, 50, and 55 percent. Sampling dates included collection of plant samples for yield and quality determination. At each sampling date, ten adjacent plants per plot randomly selected and bordering unharvested rows were harvested by handclipping. For determination of dry matter (DM) content and yield, the plants were weighed, separated into ear and stover (including husks), chopped, and representative sub-samples were oven-dried at 105°C to constant weight. Another sub-sample of both ear and stover was subsequently stored at -18°C for quality determination. After freeze-drying the samples were at first pre-ground in a rotor beater mill to pass a 4 mm sieve (Retsch GmbH, Haan, Germany) and subsequently ground in a Cyclotec mill (Foss Tecator AB, Höganäs,

Sweden) to a 1 mm particle size. In total, 480 samples of ear and 640 samples of stover were available for quality analysis.

Table 1.
Climatic conditions given as annual values and corresponding data for the maize vegetation period (1st May to 30th September) of mean temperature [°C], mean radiation [J $cm^{-2}$ $d^{-1}$], and precipitation sum [mm] for the experimental years (2001-2003) and the longterm average (1974-2005). Data were kindly provided for the nearest weather station (Kiel-Holtenau, 54° 22' N, 10° 08'E, 31 m altitude) by the German Weather Service.

| | precipitation [mm] | | temperature [°C] | | radiation [J $cm^{-2}$ $d^{-1}$] | |
|---|---|---|---|---|---|---|
| | annual | veg. period | annual | veg. period | annual | veg. period |
| 2001 | 810.3 | 436.2 | 8.8 | 14.8 | 987.9 | 1658.0 |
| 2002 | 960.9 | 445.3 | 9.7 | 16.4 | 1002.4 | 1651.3 |
| 2003 | 524.1 | 210.1 | 9.6 | 16.7 | 1070.3 | 1731.5 |
| 1974-2005 | 759.1 | 332.4 | 8.7 | 14.8 | 989.1 | 1632.2 |

*2.2 Analysis of water soluble carbohydrates*

The content of WSC was estimated using near-infrared reflectance spectroscopy (NIRS). Ground samples were scanned using a NIRSystems 5000 scanning monochromator (FOSS GmbH, Rellingen, Germany). Calibrations were developed separately for ear and stover, based on 88 ear samples and 210 stover samples, while for validation 40 samples were selected of each fraction. The WSC content of the calibration and validation subset then was determined by a modified anthrone method as described by van Handel (1967) and McAllen (1985). Equations for NIRS prediction were developed using the Infrasoft (ISI, Port Matilda, PA) software with the modified partial least squares method (Shenk and Westerhaus, 1991). We found good correlation between conventional analysis and the NIRS determined values. Coefficients of correlation were 0.99 and 0.98 for ear and stover, respectively. Standard errors of calibration and validation were below 12.6 g WSC $kg^{-1}$ DM with mean WSC contents in ear of 164.7 g $kg^{-1}$ DM (range: 21.8 – 432.8 g $kg^{-1}$ DM) and in stover of 162.9 g $kg^{-1}$ DM (range: 1.0 – 351.3 g $kg^{-1}$ DM). Whole plant contents of WSC were derived from corresponding values of stover and ear and their weight proportions.

*2.3 Statistical analysis*

A mixed model analysis for WSC concentration was calculated using PROC MIXED of SAS 8.2 (SAS Institute Inc., 2001) by considering year, maturity group, variety within maturity group, harvest date, and block as fixed factors and by assuming a heterogeneous, auto-regressive covariance structure for repeated measurements. The resulting model equation for a given *WSC* concentration was

$$WSC_{ijklm} = M + y_i + mat_j + \mathrm{var(mat)}_{jk} + har_l + bl_m + (mat * har)_{jl} + (var(mat) * har)_{jkl} + (y * mat)_{ij} + (y * var(mat))_{ijk} + (y * har)_{il} + (\mathrm{y} * \mathrm{mat} * \mathrm{har})_{ijl} + (\mathrm{y} * \mathrm{var(mat)} * \mathrm{har})_{ijkl} + e_{ijklm}$$

where M is the overall mean, $y_i$ is year *i*, $mat_j$ is maturity group *j*, $\mathrm{var(mat)}_{jk}$: variety *k* nested in maturity group *j*, $har_l$ is harvest date *l*, $bl_m$ is block *m*, and $e_{ijklm}$ denotes the residual error. Effects were considered significant in all statistical calculations for P-values < 0.05. In case of significant interactions, linear contrasts were calculated using the SLICE procedure in SAS. Comparison of means was performed by t-test with a Bonferroni-Holm adjustment.

*2.4 Model description*

The WSC content of the whole crop served as input for the weather-based dynamic model FONSCH (FOrage NonStructural CarboHydrates), originally developed to simulate the WSC concentration of temperate forage grasses. The model algorithms have been described in detail elsewhere (Wulfes et al., 1999). Briefly, the model assumes the general trend of WSC content to follow an optimum curve, which describes the indirect impact of weather conditions on WSC content via its influence on the phenological development of the crop. Environmental factors (temperature, radiation, and plant-available soil water) are converted into corresponding change rates based on corresponding exponential functions. Beforehand, the plant-available soil water is calculated in the growth part of the model. Daily environmental change rates are obtained as the product of the rates for temperature, radiation, and plant-available soil water, and are related to the trend of WSC change by an appropriate function. In addition to the general trend, day-to-day changes of WSC content are calculated, representing the direct impact of weather conditions on photosynthesis and respiration. To be able to estimate the parameters required for calculating the WSC short-term fluctuations properly, a high frequency of samplings is required throughout the growing

period. Our field study comprised 6 sampling dates, which was not considered sufficient. The calculation of day-to-day changes therefore was deactivated, and changes of WSC content described exclusively by the general trend. In the model, we assumed high temperature and solar radiation to enhance crop development and starch accumulation and thus to accelerate the intensity of quality change. Water deficiency, in contrast, may slow down crop development and therefore was supposed to decelerate WSC changes. Model calibration was conducted by an integrated parameter optimization module, which minimizes the deviation between simulated and experimental data in terms of the sum of squared residuals. Model parameters were optimized for each hybrid separately. Model fit was assessed by the coefficient of determination ($R^2$), and the root mean square error (RMSE).

*2.5 Design of the simulation study*

In North Western Europe, environmental constraints, i.e. low irradiation intensities paralleled by low temperatures and high amounts of rainfall particularly during the grain filling period, may reduce the WSC of silage maize and thus limit ensilability and aerobic stability of silages. The field study presented included three years, which probably will not comprise the expected variability of environmental conditions for the experimental site. We therefore applied the FONSCH model to conduct a 30- year (1976-2005) simulation, which allowed to comprehensively quantify the environmental impact on whole crop WSC content at silage maturity for the Kiel site, which may represent the marginal regions of silage maize production in Northern Germany and Denmark. Before running the simulation, harvest dates had been determined using the FOPROQ model (Herrmann et al., 2005, 2006) by supposing an optimum harvest at a whole crop DM content of 350 g DM $kg^{-1}$ fresh weight. For unfavorable climatic conditions with delayed maturity we set $10^{th}$ October as the latest possible harvest date if silage maturity was not reached until then. The simulation study was restricted to cultivars Oldham and Fuego, representing the early and mid-early maturity groups, respectively. The year-to-year changes of simulated WSC contents are displayed graphically and the frequency distributions are characterized by their skewness, kurtosis, mean, and the 10%, 25%, 50%, 75%, and 90% quantiles.

Table 2.

Whole plant dry matter content [g $kg^{-1}$ fresh matter] of the tested hybrids provided for the sampling dates as means of the growing periods 2001-2003.

| harvest date | Julian Day | Arsenal (early) | Oldham (early) | Symphony (early) | Probat (mid-early) | Attribut (mid-early) | Fuego (mid-early) | Clarica (mid-late) | Benicia (mid-late) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 187 | 108.05 | 99.40 | 114.77 | 103.89 | 112.20 | 107.06 | 109.44 | 95.65 |
| 2 | 230 | 207.51 | 209.09 | 223.08 | 200.86 | 212.09 | 218.82 | 197.73 | 195.52 |
| 3 | 238 | 231.75 | 223.95 | 252.96 | 220.52 | 233.89 | 246.72 | 216.05 | 221.97 |
| 4 | 246 | 293.68 | 288.68 | 303.53 | 270.21 | 276.20 | 285.92 | 256.06 | 256.21 |
| 5 | 259 | 347.66 | 322.05 | 335.12 | 321.93 | 319.86 | 316.13 | 284.66 | 288.39 |
| 6 | 276 | 393.15 | 407.09 | 391.41 | 388.36 | 385.13 | 369.13 | 333.63 | 327.79 |

## 3. Results

### *3.1 Water soluble carbohydrate content of ear, stover and whole plant*

The samplings conducted in the present study covered a wide range of developmental stages of the maize crop, as suggested by the whole crop DM content (Tab. 2). Silage maturity was achieved at harvest 5 for the early and mid-early maturity group, and at harvest 6 for the mid-late group, if we assume a DM content of 320 g $kg^{-1}$ fresh matter as criteria of silage maturity. Due to the large variation in development, harvest date probably represented the largest component of variance (Tab. 3). Significant effects of the year and its 2-way interactions document that apart from harvest date, environmental conditions influenced WSC content considerably. The impact of genotype became apparent in significant interactions of variety within maturity x harvest date and maturity x harvest date for all plant fractions considered. The 3-way interaction year x harvest date x maturity was generally significant. Linear contrasts, performed by the SAS slice option in Proc MIXED, however, showed similar effects in each year. In the following, we will focus on the impact of genotype on WSC content, while the impact of weather conditions will be presented in detail in the section on modeling.

Table 3.
Influence of the factors 'year', 'maturity group' (mat.), 'variety (var.) within maturity group' 'harvest date (har.date)' and 'block' on the contents of NDF and ADF of whole-plant, stover and ear. Statistical analysis.

| ***WSC*** | Num DF | | | F Value | | |
|---|---|---|---|---|---|---|
| Effect | ear | stover | whole-plant | ear | stover | whole-plant |
| year | 2 | 2 | 2 | 124.98 *** | 2.55 n.s. | 21.12 *** |
| mat. | 2 | 2 | 2 | 128.95 *** | 102.97 *** | 136.76 *** |
| var. (mat.) | 5 | 5 | 5 | 23.29 *** | 16.88 *** | 16.68 *** |
| harvest date | 4 | 5 | 5 | 1489.52 *** | 545.87 *** | 1204.04 *** |
| block | 1 | 1 | 1 | 5.44 * | 2.51 n.s. | 7.03 * |
| mat.*har. date | 8 | 10 | 10 | 17.18 *** | 22.03 ** | 22.94 *** |
| var. (mat.)*har. date | 20 | 25 | 25 | 4.21 *** | 2.45 ** | 2.69 * |
| year*mat. | 4 | 4 | 4 | 6.41 ** | 1.54 n.s. | 3.01 * |
| year*var. (mat.) | 10 | 10 | 10 | 1.61 n.s. | 1.75 n.s. | 1.64 n.s. |
| year*har. date | 8 | 10 | 10 | 75.26 *** | 25.96 *** | 48.63 *** |
| year*har. date*mat. | 16 | 20 | 20 | 4.72 *** | 2.71 * | 4.39 *** |
| year*har.date*var.(mat.) | 40 | 50 | 50 | 1.58 n.s. | 1.05 n.s. | 1.39 n.s. |

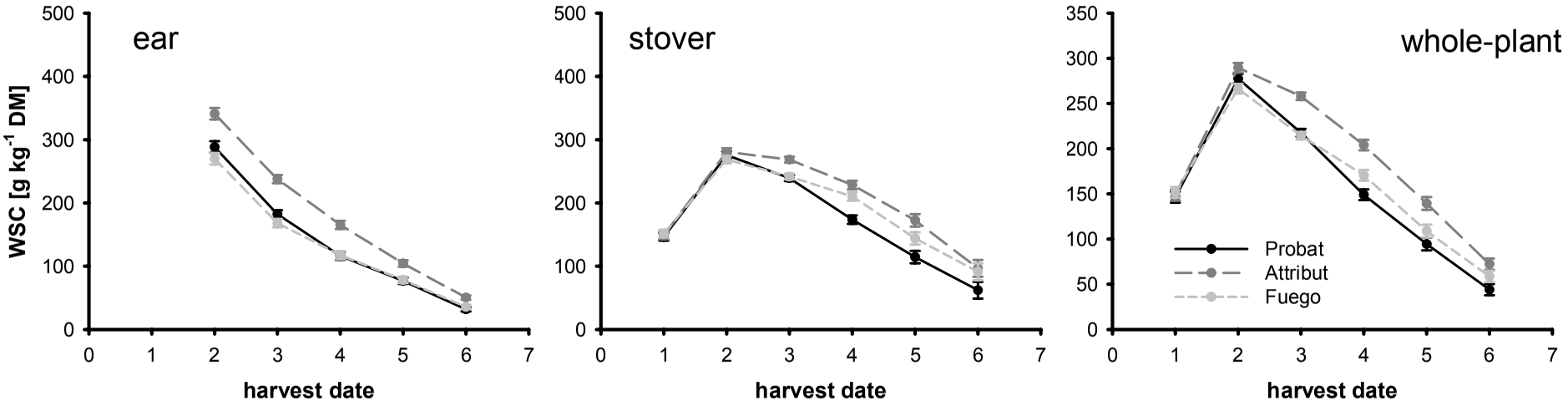


Figure 1.

Content of water soluble carbohydrates in ear, stover, and whole crop of the varieties belonging to the mid-early maturity group, displayed as mean of three experimental years (2001-2003). Bars around points indicate ± standard error (SE); where bars are not shown, points were larger than the SE.

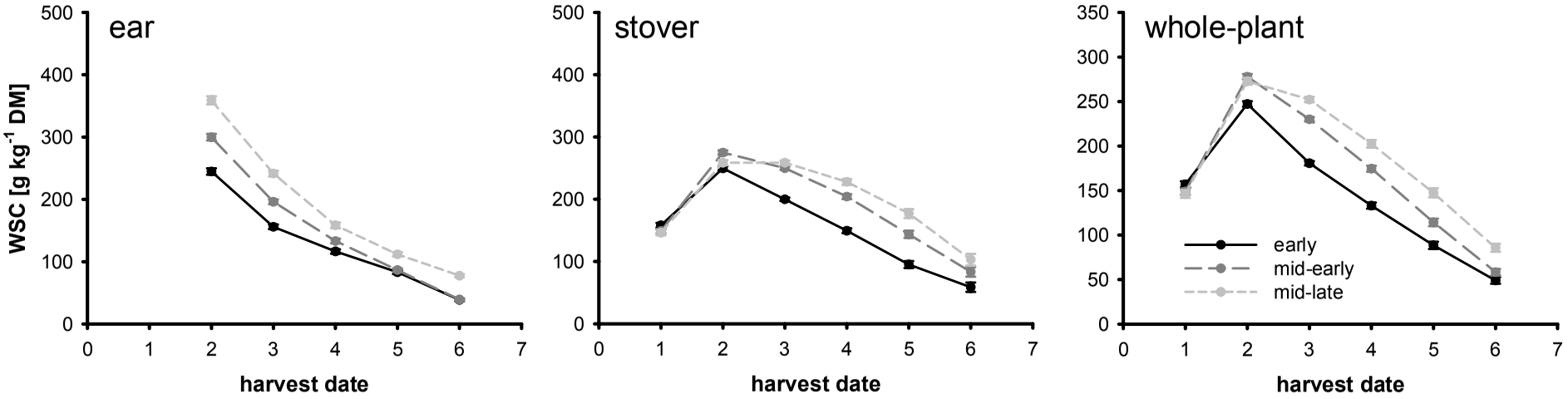


Figure 2.

Content of water soluble carbohydrates in ear, stover, and whole crop of the early, mid-early, and mid-late maturity group, displayed as mean of three experimental years (2001-2003). Bars around points indicate ± standard error (SE); where bars are not shown, points were larger than the SE

Differences among varieties within maturity group were most pronounced for the mid-early group, where Attribut exceeded Probat and Fuego significantly at most harvest dates following silking, i.e. between harvest dates 3 to 5 (Fig. 1). At harvest date 6 variation among varieties diminished. In the early group, variety Arsenal was characterized by significantly higher stover and whole crop WSC contents at harvest dates 2 to 4 (data not shown), amounting to a maximum difference of 44.1 g WSC $kg^{-1}$ DM in stover (harvest date 3) and 28.5 g WSC $kg^{-1}$ DM in the whole crop (harvest date 2). This is surprising, since Arsenal represents a maize type maturing fast in stover. In the mid-late group, significances were detected in the ear fraction at dates 2, 3, and 6 only, with higher values for Benicia (not presented). We can conclude from the data that the variation in WSC content is largest in the grain filling period. At silage maturity, a varietal impact was detected in the mid-early group only, with a maximum difference of 44.8 g WSC $kg^{-1}$ DM.

The interaction maturity x harvest date is presented in Fig. 2. The ear WSC contents showed a steady decline over the growing season, ranging between 206.1 g WSC $kg^{-1}$ DM for the early and 281.5 g $kg^{-1}$ DM for the mid-late group. WSC contents differed significantly at all sampling dates between the early and the mid-late as well as the mid-early and mid-late group. Largest differences among the maturity groups were detected at harvest date 2, where the mid-late group exceeded the early group by 114.2 g WSC $kg^{-1}$ DM. With advancing maturity, the differences decreased, amounting to 28.7 g $kg^{-1}$ DM at harvest date 5 and 38.8 g WSC $kg^{-1}$ DM at harvest 6. In stover, the seasonal changes in WSC reflect the typical accumulation and remobilization of carbohydrate reserves in the stem, with increasing contents before and a clear decline after silking. In contrast to the ear, variation among maturity groups was most pronounced at harvest date 5, with a difference of 82.0 g WSC $kg^{-1}$ DM between the early and the mid-late group. The early group showed significantly higher concentrations compared to the mid-early and mid-late group throughout harvest dates 3 to 5. At harvest 6, however, no significant variation was detected. Changes of whole crop WSC concentration over the growing season are similar to that of the stover fraction. After silking, contents decreased by 187.0 (mid-late) to 220.0 g WSC $kg^{-1}$ DM (mid-early). Maximum WSC contents, observed at harvest date 2, were significantly higher for the mid-early and mid-late group compared to the early varieties. Differences among groups remained considerable,

being significant at harvest dates 3 to 5, while at harvest 6 only the early and mid-late group showed a substantial difference of 36.6 g WSC $kg^{-1}$ DM.

*3.2 Modeling of whole crop WSC content*

We restrict the presentation of model calibration to varieties Oldham and Fuego, representing the early and mid-early group. Other hybrids reacted in a similar manner and calibration resulted in comparable goodness of model fit.

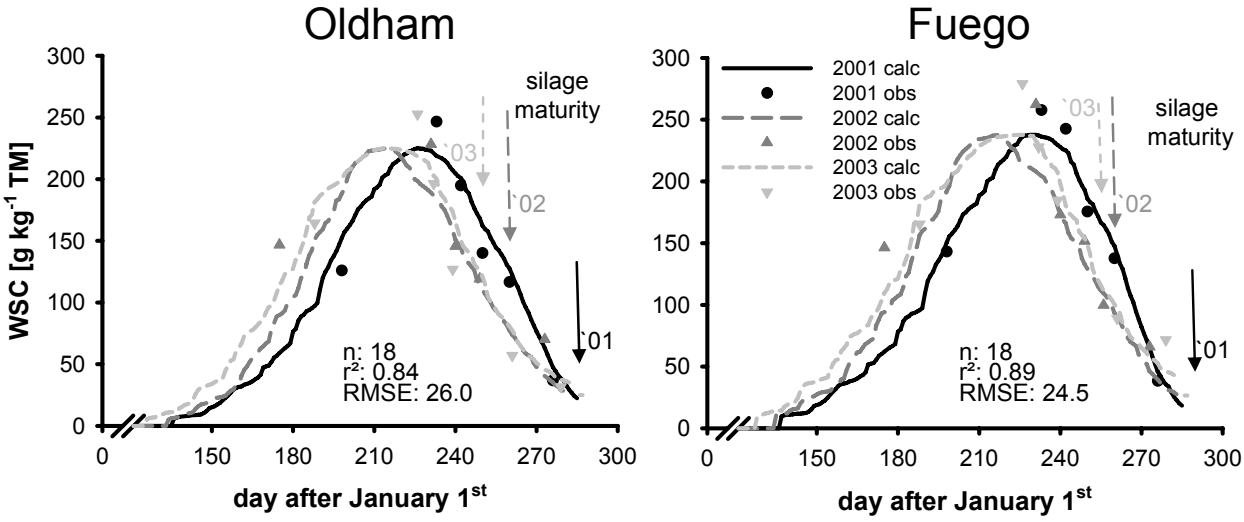


Figure 3.
Observed (symbols) and calculated (lines) data of water soluble carbohydrates (WSC) in the whole-plant of the varieties Oldham and Fuego (2001-2003). Arrows indicating silage maturity in corresponding years (~320 g DM $kg^{-1}$).

Fig. 3 displays the simulated and observed whole crop WSC contents. Low temperature and radiation in the growing period 2001 caused a slower crop development. Compared to 2002 and 2003, silage maturity was delayed by about 30 days in 2001 and resulted in a substantially lower WSC content. Growing periods 2002 and 2003 showed minor differences in harvest date and WSC content, although 2003 was characterized by severe drought conditions after silking (Tab. 1).

Model calibration concerned a total of 54 parameters, 28 originating from the growth part and 26 from the quality part relating to the general trend of WSC change. Out of the growth parameters, 25 were assumed identical for both varieties, while three parameters were

estimated variety-specifically. Likewise, only four out of the 26 quality parameters required variety-specific values, namely parameters $r_c$, *ach, bch,* and *cch* (Tab. 4).

Table 4.
Results of parameter optimization of the FONSCH quality model for varieties Oldham (early) and Fuego (mid-early), based on experimental years 2001-2003.

| | | Oldham | Fuego |
|---|---|---|---|
| Growth-model | $W_0$ | 0.493 | 0.540 |
| | $r$ | 0.173 | 0.175 |
| | $a$ | 2.00 | 1.90 |
| Quality model: WSC content | $t_c$ | 0.012 | 0.012 |
| | $T_{th}$ | 6.0 | 6.0 |
| | $r_c$ | 0.114 | 0.139 |
| | $R_{th}$ | 3.0 | 3.0 |
| | $m_c$ | 0.001 | 0.001 |
| | *ach* | 10.54 | 53.001 |
| | *bch* | 9.10 | 10.262 |
| | *cch* | 3.08 | 4.082 |

with $W_0$: aboveground biomass at onset of growth, r : relative growth rate at onset of growth, a : constant determining the curvature of the function for LAI calculation, $t_c$ : constant of temperature change function, $T_{th}$ : temperature threshold in temperature change function, $r_c$ : constant of radiation change function, $R_{th}$ : radiation threshold in radiation change function, $m_c$ : constant in soil moisture change function; *ach, bch, cch* : constants determining the curvature of function, which relates the accumulated daily environmental change rates to the WSC content

Model optimization confirmed the major impact of temperature and radiation on the intensity of WSC change. The parameters, determining the shape of the temperature response function ($t_c$, $T_{th}$), could be assigned uniformly. For solar radiation cultivar-specific values gave better agreement with measured data for parameter $r_c$ (shape of radiation response function), indicating a slightly higher sensitivity for Fuego. Parameter $R_{th}$, representing a threshold radiation value, however, revealed no differences between varieties. Plant available soil water turned out to essentially have no impact on WSC content. Simulated and observed WSC concentrations generally agreed well for both cultivars, explaining 84 to 89% of the observed variation (Fig. 3). Prediction errors ranged between 24.5 and 26.0 g WSC $kg^{-1}$ DM.

Consistently, larger deviations between measured and calculated values were observed in the pre-silking phase up to the point of maximum WSC content, especially in the experimental year 2002. Maximum values generally were underestimated by the FONSCH model. In the post-silking phase, WSC content was reasonably well estimated for both cultivars. Simulated WSC contents at silage maturity document a comparatively high impact of environmental conditions, with a difference of 80 to 100 g WSC $kg^{-1}$ DM between growing seasons 2001 and 2003 for Oldham and Fuego, respectively. The impact of variety, in contrast, seems less important, amounting to about 35 g WSC $kg^{-1}$ DM.

*3.3 Long-term simulation of WSC content*

The results of the 30-year (1976-2005) simulation study are displayed over time (Fig. 4) and as frequency distribution (Fig. 5), and corresponding statistics are provided in Table 5.

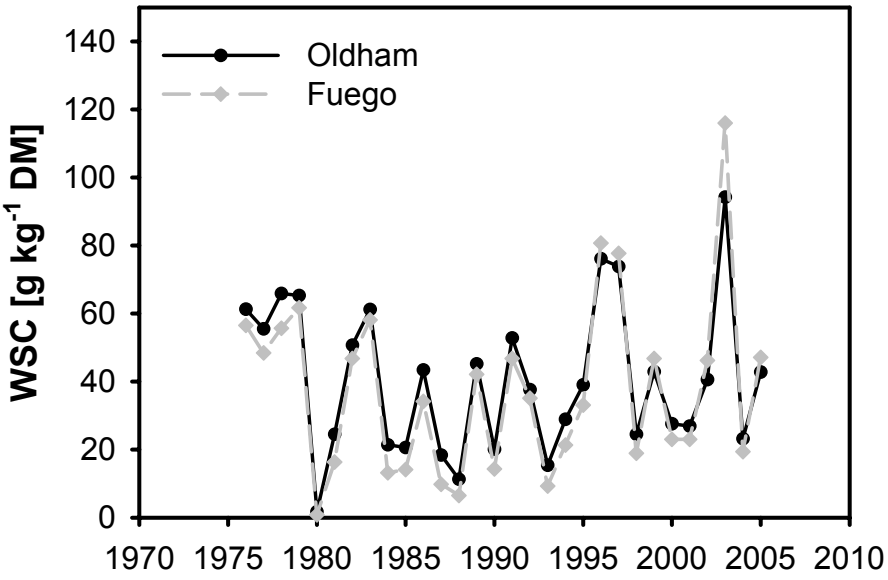


Figure 4.

Results of the simulation experiment (1976-2005) given as time series for the content of water soluble carbohydrates in the whole-plant of the varieties Oldham and Fuego.

Table 5.

Results of the simulation experiment (1976-2005), given as minimum (min) and maximum (max) value, skewness, kurtosis, median and mean value, and as coefficient of variation (cv [%]) for the content of WSC in the whole-plant of the varieties Oldham and Fuego.

| | *min* | *max* | *mean* | *median* | *cv [%]* | *skewness* | *kurtosis* |
|---|---|---|---|---|---|---|---|
| ***Oldham*** | 2.0 | 94.0 | 4.0 | 4.0 | 54.1 | 0.5 | -0.2 |
| ***Fuego*** | 1.0 | 116.0 | 3.7 | 3.5 | 68.9 | 1.0 | 1.5 |

The dispersion of the distribution was characterized by the coefficient of variation (cv), the excess kurtosis, and the skewness. Positive kurtosis values indicate a peaked distribution and negative values a flat distribution, i.e. a risk of high instability. Negative values for the skewness suppose that data are skewed left, while positive values indicate right skewness. Year-to-year variation of the whole crop WSC content was substantial and consistent for both varieties. Highest concentrations were found for the 2003 growing season (94 and 116 g WSC $kg^{-1}$ DM for Oldham and Fuego, respectively), which was characterized by high temperature and irradiation, as well as drought conditions.

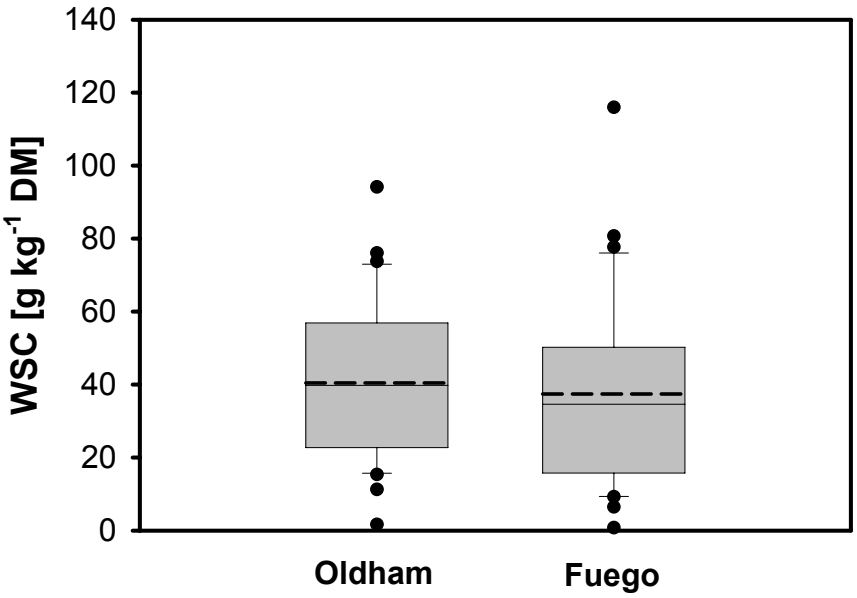


Figure 5.

Results of the simulation experiment (1976-2005) given as frequency distribution of content of water soluble carbohydrates for the varieties Oldham and Fuego. Boxes: 25, 50 and 75% quantiles, mean value (---); whisker caps: 10 and 90% quantiles, and outliers (●).

Lowest values occurred in 1980 (< 1 g WSC $kg^{-1}$ DM), which had below average solar radiation during the vegetation period (1389.6 J $cm^{-2}$), but above average temperature (15.9°C). In 80 percent of the years included in the study, the early variety Oldham exceeded mid-early Fuego in WSC content.

Largest differences between the varieties were detected in 2003, where concentration of Fuego was 22 g $kg^{-1}$ DM higher compared to Oldham. On average, however, the difference between the varieties was marginal, amounting to 3 g WSC $kg^{-1}$ DM. The analysis of the frequency distributions confirms left-skewed distributions for both varieties, i.e. a high abundance of years with low WSC content, as supposed by positive skewness values. The mid-early Fuego, however, is characterized by a higher sensitivity, as indicated by a larger coefficient of variation, which is also confirmed by a positive kurtosis.

## 4. Discussion

### *4.1 Seasonal changes of WSC content*

In the present study substantial changes of WSC content in whole crop, stover, and ear were recorded throughout the vegetation period, which are in good agreement with previous studies (McAllan and Phipps, 1977; Phipps and Weller, 1979; Phipps et al., 1984; Oldenburg and Laws, 1993). Increasing concentrations until silking and decreasing WSC contents afterwards reflect the changing source-sink ratios. Typically, WSC are accumulated by photosynthesis and stored for the most part in stems and leaves of the maize plants under optimal growing conditions (Phipps et al., 1984). After silking, newly synthesized and remobilized assimilates (primarily sucrose) are translocated to the developing kernels (Hawker et al., 1991; Kühn et al., 1999). At high plant density (15 plants $m^{-2}$), Mc Allen and Phipps (1977) reported ear and stover WSC contents of 97 and 102 g WSC $kg^{-1}$ DM at the end of the sampling period, respectively, which coincides with our results. Irlbeck et al. (1993) detected a slightly lower content of total non structural carbohydrates in stover (59 to 68 g $kg^{-1}$ DM). In the whole crop, we observed the maximum concentration at harvest date 2 to vary between 247.2 and 277.9 g WSC $kg^{-1}$ DM. Comparative studies are scarce and inconsistent. Our findings closely match those obtained by Oldenburg and Laws (1993), who reported WSC to range between 230-280g $kg^{-1}$ DM 6 to 8 weeks before silage maturity. Andrieu et al. (1993), analyzing 234 maize samples varying in DM content between 183 to

531 g DM $kg^{-1}$ fresh material, found WSC contents of 36-226 g WSC $kg^{-1}$ DM. McAllen and Phipps (1977), in contrast, detected substantially higher WSC concentrations of 325.9 g WSC $kg^{-1}$ DM around flowering. The apparent discrepancies may be due to fact, that probably none of the studies managed to find the “true” maximum WSC content.

*4.2 Genotypic impact on WSC content*

The whole crop WSC content at silage maturity was reported to range between 80 and 101 g WSC $kg^{-1}$ DM, depending on genotype, plant density and grain fill (Phipps et al., 1984; Wilkinson and Phipps, 1979). Our study showed a wider range between 77.5 and 139.5 g WSC $kg^{-1}$ DM among all varieties investigated. This may be due to the larger number of varieties included. Furthermore, our sampling schedule did not provide for harvesting the maturity groups at comparable development stages. Varieties belonging to the early group were somewhat further advanced in maturity at harvest 5 (assumed silage maturity for early and mid-early varieties) than the mid-early group, and mid-late varieties experienced a different environment until reaching silage maturity at harvest 6. Differences among varieties within maturity group at silage maturity were significant only in the mid-early group, where variety Attribut turned out to have significantly higher WSC content. The difference amounted to 44.8 g WSC $kg^{-1}$ DM. It seems likely that this is attributable to the genetical background. In our study, Attribut was the only pure flint type maize included, which is known to have a lower ear-to-stover ratio compared to dent types (Phillipeau and Michalet-Doreau, 1997). An analysis of variance for the ear-to-stover ratio confirmed this assumption (data not shown). Support for our findings comes from the study of Andrieu et al. (1993), who investigated the forage quality of 15 varieties and found genotype to have only a very little effect on WSC concentration. Similarly, Irlbeck et al. (1993) reported that the content of total non-structural carbohydrates of the stover was not influenced by genotype. It might be argued, that the number of varieties included in our study does not allow to draw a general conclusion on genotypically caused variation of WSC content. The varieties, however, were specifically selected to represent the spectrum of silage maize types grown in Germany (Herrmann et al., 2006).

*4.3 Environmental impacts on WSC contents*

The results of the present study revealed a substantial impact on WSC content of the ear, the stover, and the whole crop. The WSC concentration observed in the growing period 2003 exceeded the values recorded in 2002, but especially the contents of 2001 (Fig. 3). One possible theory for this finding is a reduced sink strength of the kernels, caused by adverse environmental conditions around silking (Classen and Shaw, 1970; Setter et al., 2001). It is widely accepted, that maize is highly susceptible to drought conditions at flowering (Grant et al, 1989). A reduced sink strength of kernels, however, would have resulted in a lower ear-to-stover ratio in the growing period 2003, which is not supported by our data (not presented). Model calculations furthermore indicate that severe water shortage started after silking (Fig.6).

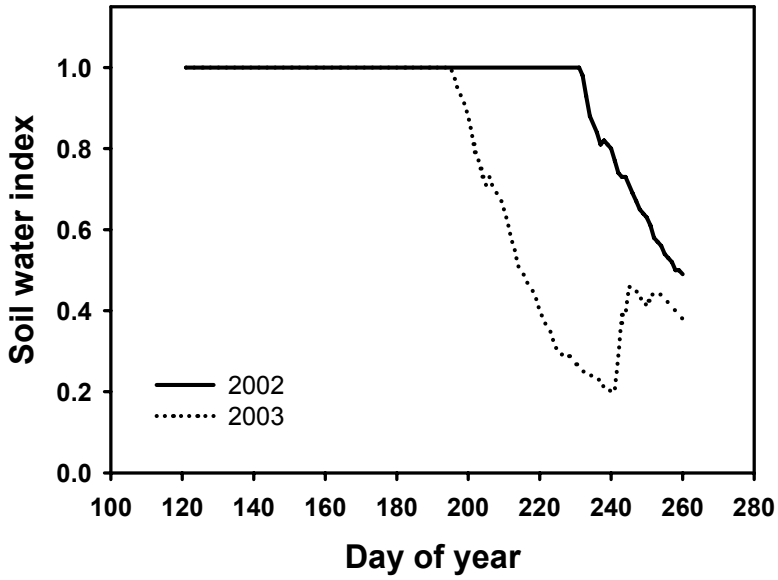


Figure 6.
Calculated soil water index of growing seasons 2002 and 2003, exemplied for cultivar Oldham. The soil water index, ranging between 0 and 1, denotes the ratio of actual to potential plant available soil water.

A further possible explanation is an enhanced maturation of stover, which limited the remobilization and translocation of assimilates from the stover to the ear (Westgate, 1994). We then would expect an increase of WSC content in stover, and a decrease in the ear. In fact, we find a higher WSC content at a comparable DM content for the stover in 2003, but not for the ear (Fig. 7). Finally, high temperature and radiation in the experimental year 2003,

may have promoted photosynthetic activity without exhausting the carbohydrate reserves in the stover and the ear. Probably, both processes took effect. Model calibration showed a large impact of temperature and solar radiation on WSC content. The soil water availability turned out to have essentially no influence on the change of WSC, which supports the afore stated hypothesis of increased photosynthetic activity. Overall, there was a good agreement between observed and calculated WSC concentrations, as indicated by coefficients of correlations above 0.84 (Fig. 3). The FONSCH model proved its general suitability to predict WSC contents not only for forage grasses, but also for forage maize without any modification of model algorithms. The applicability of the current version, however, is limited if severe stress conditions at silking result in incomplete ear pollination, ear barrenness, and kernel abortion, and by extent increasing WSC contents with advancing maturity, as reported in several studies (Tollenaar, 1977; Phipps et al., 1984; Uhart and Andrade, 1995).

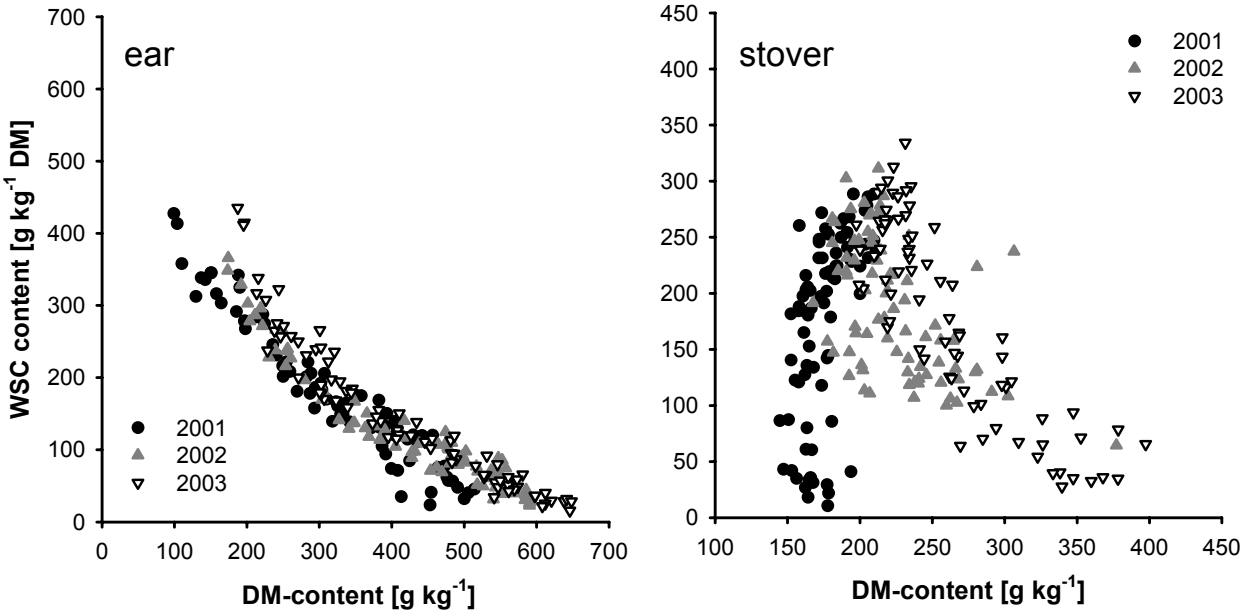


Figure 7.
Content of water soluble carbohydrates against dry matter content of ear and stover samples for the years 2001-2003. Symbols represent block measurement.

Larger deviations between measured and calculated WSC contents were observed rather in the pre-silking phase and around silking than in the post-silking growth period. It seems likely, that this was mainly due to the lower sampling frequency in the earlier growth stages. Model performance thus might be enhanced by enlarging the data base especially in this

respect. Furthermore, the implementation of functions for temperature and radiation separate for different developmental stages (Stewart et al., 1998), as for instance the pre- and post-silking phase, might additionally improve model performance.

The simulation study allowed a more comprehensive assessment of environmental impact on WSC content of the whole crop, revealing a substantial variation among years, with WSC concentrations varying between 1.0 and 116.0 g WSC $kg^{-1}$ DM. Compared to cell wall constituents (cv of 3.6 and 8.9 for Oldham and Fuego, Kruse et al., 2006), the WSC content (cv of 54.1 and 69.9) showed a much higher sensitivity to weather conditions, highlighting the great dependency of photosynthetic activity on environmental conditions. The impact of genotype, in contrast, was less important. There are few large scale studies on the contribution of genotype and environment on WSC content. Andrieu et al. (1993) reported the environmental variation to be more than 2-fold higher than genotypic variability, which confirms the outcome of our simulations. Yet, their work was based on two years only.

The results of the simulation study raise a further question about the assessment of the simulation output with respect to the ensiling potential of the maize crop. The fermentation potential of a crop can be rated according to the contents of DM, WSC, nitrate, and its buffering capacity (Kaiser et al., 2002). Due to interdependencies, it is difficult to provide a lower and upper WSC threshold for successful ensiling. Generally, silage maize is assumed to be easily fermentable. WSC concentrations below 3 percent in 14 out of 30 years, however, may indicate a risk of limited ensiling potential. Yet, we have to take into consideration that our data were obtained from plants harvested with a cutting height of 2-3 cm. Compared to a common cutting height of 20 cm applied on practical farms, our WSC data have a lower level.

## 5. Conclusions

Variation in ear, stover, and whole crop WSC content among varieties and maturity groups, which was more pronounced in early grain fill, reflect the differences in maturation behavior and source-sink relationships. Significant differences in the mid-early maturity group at silage maturity probably can be traced back to the genetic background, i.e. the generally lower ear-to-stover ratio of flint compared to dent type maize varieties. The FONSCH model, originally developed for forage grasses, turned out to be applicable to forage maize without any

modifications of its algorithms. Although we deactivated the functions for the daily fluctuations of WSC and used the general trend only, the experimentally obtained WSC concentrations were reproduced with acceptable accuracy. The application of the FONSCH model allowed to replace the few meaningful factor year by the specific factors temperature, solar radiation, and plant available soil water (precipitation), and to estimate their contribution to WSC change. The field experiment together with the results of the simulation study revealed a substantially larger contribution of environmental conditions than genotype on the WSC concentration at silage maturity. Moreover, for the environmental conditions of Northern Germany there seems to be a higher risk of too low than to high WSC concentrations. Further work will comprise the validation of the FONSCH model.

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# Kapitel 5

# Generaldiskussion

*5.1 Einleitung*

Die Silomaisproduktion nimmt in Europa und in Deutschland einen großen Stellenwert ein. Nicht nur die Wiederkäuerernährung sondern auch alternative Nutzungsmöglichkeiten, beispielsweise die Fermentation von Silomais in Biogasanlagen, erfordern die Bereitstellung optimaler Qualitäten, um das genetische Potential der Nutztiere einerseits und das Potential der Energiegewinnung andererseits voll ausschöpfen zu können.

Die momentane Charakterisierung von Silomaissorten erfolgt neben Anbaueigenschaften weitestgehend über das Ertragspotential und wird durch einzelne Qualitätsparameter, wie u. a. dem Stärke-, Rohfaser-, Rohprotein- und Energie-Gehalt, ergänzt (Anonymus, 2006a, b). Auf Grundlage des Trockensunbstanz- (TS-) Gehaltes der Gesamtpflanze werden entsprechende Silomaissorten weiterhin in nutzungsspezifische Reifegruppen eingeordnet (Miltner und Rath, 1998).

Probleme hinsichtlich der genauen Beschreibung von Silomaissorten bestehen jedoch dahingehend, dass sowohl der Qualitätsentwicklung in der Vegetationsperiode ebenso wenig Rechnung getragen wird, wie den unterschiedlichen Verläufen der Qualitätsentwicklung im Kolben und in der Restpflanze und ihren entkoppelten Abreifen, z. B. bei stay green bzw. dry down Sorten. Insbesondere werden die verschiedenen Struktur- und Nicht-Struktur-Kohlenhydrate, die spezifische Einflüsse auf den energetischen Futterwert von Silomais ausüben, nicht zur Beschreibung von Silomaisgenotypen herangezogen. Eine adäquat differenzierende analytische Charakterisierung der Inhaltsstoffe von Futtermitteln und dabei insbesondere eine genauere Erfassung der Kohlenhydratfraktionen wird indes als essentiell für eine Futterwertbestimmung angesehen (Südekum, 2001). Dieses kann mit Hilfe der Nah-Infrarot-Reflexions-Spektroskopie (NIRS) erfolgen, die zur Bestimmung von chemischen Inhaltsstoffen von Futtermitteln ergänzend zu den beschriebenen Standarduntersuchungen etabliert ist (Murray, 1993; Givens und Deaville, 1999; Stuth et al., 2003). Die Schätzgenauigkeit der NIRS zur Bestimmung der Gasbildung in Anlehnung an den Hohenheimer Futterwerttest erweist sich dagegen bisher als ungenügend (Lovett et al., 2004).

Neben der genauen analytischen Beschreibung der Qualitätsparameter im Zuwachsverlauf stellt die Prognose des optimalen Erntezeitpunktes unter Berücksichtigung der Witterung derzeit eine weitere Herausforderung zur Steigerung des Futterwertes in der Wiederkäuerernährung dar. Adäquate Simulationsmodelle bieten die Möglichkeit, die Qualitätsentwicklung von Silomais auf Basis leicht verfügbarer Wetterdaten zu kalkulieren und bei

entsprechend vorliegenden Kalibrationen, Managemententscheidungen bezüglich des Erntezeitpunktes auch auf Grundlage spezifischer Qualitätsansprüche zu unterstützen.

*5.2 Zusammenfassende Darstellung der Untersuchungsergebnisse*

Vor der oben erläuterten Problemstellung war das übergeordnete Ziel dieser Arbeit, eine genaue Charakterisierung von Silomaisgenotypen anhand futterwertbestimmender Parameter im Vegetationsverlauf vorzunehmen und die genotyp- bzw. umweltbedingte Variation zu quantifizieren.

Die Qualitätsentwicklung im Vegetationsverlauf wurde an acht Silomaissorten, die im Hinblick auf Reifegruppe (früh, mittelfrüh, mittelspät), Abreifeverhalten (normal, stay green, dry down) und Inhaltsstoffzusammensetzung das Sortenspektrum repräsentieren, bestimmt. Eine Analyse der Gehalte an Nicht-Struktur-Kohlenhydraten (Stärke und wasserlösliche Kohlenhydrate (WLK)) und Zellwandbestandteilen (neutral detergent fiber (NDF), acid detergent fiber (ADF), Hemicellulose, Cellulose und Lignin) erfolgte in den Kolben, Rest- und Gesamtpflanzen zu 6 Terminen (1 vor, 5 nach der Blüte) innerhalb der Vegetationsperiode. Die Bestimmung der genotypbedingten Variation wurde mittels Varianzanalyse vorgenommen; Witterungseinflüsse wurden mit Hilfe der Modelle FOPROQ (Kornher et al., 1991; Herrmann et al., 2005) und FONSCH (Wulfes et al., 1999) quantifiziert.

Weiteres Versuchsziel war es zu prüfen, ob durch die getrennte Betrachtung von Kolben und Restpflanze bzw. durch die Bestimmung von in definierten Inkubationsintervallen gebildeten Gasvolumina, die NIRS-Schätzgenauigkeit bezüglich der Gasbildung gesteigert werden kann.

In Kapitel 2 wurde dieser letzte methodische Aspekt ausführlich behandelt.

Als zentrales Ergebnis bleibt festzuhalten, dass trotz der Fraktionierung der Mais-Gesamtpflanze in Kolben und Restpflanze und der Bestimmung der Gasvolumina einzelner Inkubationsintervalle, die mit der Fermentation verschiedener Kohlenhydratfraktion in Beziehung stehen, keine sicheren NIRS-Schätzgleichungen aufgestellt werden konnten. Obgleich eine Verbesserung der Kalibrationsgüte im Vergleich zu anderen Studien dokumentiert wurde, stellte sich die Validationsstatistik für alle untersuchten Inkubationsintervalle als unbefriedigend dar. Schwierigkeiten bezüglich der Reproduzierbarkeit der Gasbildungsergebnisse im Labor könnten ebenso wie die zwar in verschiedenen Raten, trotzdem aber gleichzeitig ab-

laufende Fermentation einzelner Kohlenhydratfraktionen, Fehlerquellen darstellen, die eine exakte Schätzung verhindern.

In Kapitel 3 wurden die untersuchten Sorten bezüglich ihres NDF-, ADF-, Hemicellulose-, Cellulose- und Lignin-Gehaltes charakterisiert und die umweltbedingte Variation bestimmt. Unterschiede zwischen den Sorten konnten statistisch sowohl in der Restpflanze, dem Kolben als auch in der Gesamtpflanze abgesichert werden. Jedoch zeigte sich bei näherer Analyse dieser Unterschiede, dass sich die Sorten innerhalb der jeweiligen Reifegruppe in der Gesamtpflanze im ernterelevanten Entwicklungsstadium bzw. zum Ende der Vegetationsperiode nicht signifikant in ihren Gehalten an Gerüstsubstanzen unterschieden, wohingegen signifikante Unterschiede zwischen den Reifegruppen festgestellt werden konnten. Daher sind genotypbedingte Differenzen eher auf unterschiedliche Abreifen als auf sortenspezifische Unterschiede innerhalb der Reifegruppen zurückzuführen.
Das Modell FOPROQ bewies seine Eignung, aufgrund von Wetterdaten die Qualitätsentwicklung von Silomais zu simulieren. Der signifikante Jahreseffekt konnte durch unterschiedliche Umwelteinflüsse quantifiziert werden, wobei der Temperatur und der Einstrahlung der größte Einfluss auf die Entwicklung eingeräumt wurde. Die Verläufe von NDF, ADF, Hemicellulose und Cellulose in Rest- und Gesamtpflanze konnten mit hohen Bestimmtheitsmaßen und geringen Fehlern kalkuliert werden. Sortenunterschiede in den Gerüstsubstanzgehalten sind als marginal im Vergleich zu witterungsbedingten Unterschieden einzustufen. Dieses wird ferner durch die abschließende Simulationsstudie der Gerüstsubstanzgehalte in der Gesamtpflanze zur Siloreife unter Berücksichtigung 30-jähriger Witterungsdaten belegt.

Die Ergebnisse bezüglich der Quantifizierung von genotyp- bzw. umweltbedingter Variation der Gehalte an wasserlöslichen Kohlenhydraten wurden in Kapitel 4 dargestellt.
Auch hier konnten die in früheren Entwicklungsstadien ermittelten Sortenunterschiede in Kolben, Rest- und Gesamtpflanze innerhalb der Reifegruppe in der Gesamtpflanze zur Siloreife nur begrenzt in der mittelfrühen Reifegruppe abgesichert werden. In der Gesamtpflanze wurden nach der Blüte signifikante Reifegruppenunterschiede festgestellt. Die Quantifizierung des Einflusses der Tagesdurchschnittstemperatur, Einstrahlung und des pflanzenverfügbaren Bodenwassers zeigte auch bei der Modellierung der WLK-Gesamtpflanzengehalte mittels FONSCH gute Modellanpassungen. Sortenunterschiede sind im Vergleich zu den

festgestellten Jahresschwankungen als marginal einzustufen. Die Sensitivität von WLK-Gehalten auf Witterungseinflüsse ist im Vergleich zu den Schwankungen der Gerüstsubstanzgehalte wesentlich ausgeprägter.

*5.3 Charakterisierung von Silomaisgenotypen anhand futterwertbestimmender Parameter*

Die Charakterisierung der Silomaisgenotypen erfolgte bereits eingehend durch die Gehalte an Gerüstsubstanzen (NDF, ADF, Hemicellulose, Cellulose und Lignin, Kapitel 3) und wasserlöslichen Kohlenhydraten (Kapitel 4). Der ernährungsphysiologische Wert von Silomais wird ferner erheblich durch den Stärkegehalt beeinflusst.

Zur vollständigen Charakterisierung der Kohlenhydratfraktionen werden daher nachfolgend die Stärkegehalte, die ebenfalls im Rahmen der hier vorliegenden Untersuchung erhoben wurden, dargestellt.

Im Anschluss wird das untersuchte Maissortiment außerdem anhand der morphologischen Zusammensetzung und der Abreife beschrieben, da diesen Merkmalen erhebliche Bedeutung im Hinblick auf die Entwicklung der Qualitätsparameter zukommt.

*5.3.1 Charakterisierung von Silomaisgenotypen anhand der Stärkegehalte*

Stärke, als leicht verfügbare Energiequelle, wird von Wiederkäuern vornehmlich im Pansen abgebaut. Der Vorteil von Silomais im Vergleich zu anderen Getreidestärken liegt in dem relativ hohen Anteil sogenannter By-Pass Stärke, die den Pansen unabgebaut passiert und im Dünndarm absorbiert wird (Vearasilp, 1986; Owens et al., 1986). Die verminderte Energiefreisetzung im Pansen verhindert die Gefahr der Pansenübersäuerung (Pansenacidose), so dass u. a. die Abbaubarkeit der Gerüstsubstanz positiv beeinflusst wird (De Visser et al., 1998; Owens et al., 1998). Eine hohe Anflutung von Glucose am Dünndarm birgt jedoch das Risiko höherer Glucoseverluste bei limitierter Glucoseaufnahmefähigkeit des Dünn- bzw. Dickdarms (Nocek und Tamminga, 1991; De Visser et al., 1998), weswegen die am Dünndarm anflutende Glucosemenge von 1,5 kg $d^{-1}$ nicht überschritten werden sollte (Matthé, 2000). Neben der technologischen Aufbereitung der Maissilage wird die Verdaulichkeit der Maisstärke durch die Abreife der Gesamtpflanze und die Korntextur (Philippeau und Michalet-Doreau, 1997; Johnson et al., 1999; Jensen et al., 2005) beeinflusst.

*5.3.1.1 Charakterisierung von Silomaisgenotypen anhand der Stärkegehalte*

*- genotypbedingte Variation*

Die varianzanalytische Verrechnung der Stärkegehalte in Kolben, Restpflanze und Gesamtpflanze zeigt einen signifikanten Einfluss des Faktors 'Jahr' als Haupteffekt und in Wechselwirkung (Tab. 1). Die Interaktion 'Sorte (innerhalb Reifegruppe) x Erntetermin' zeigt ebenfalls einen signifikanten Einfluss auf die Gehalte aller betrachteten Pflanzenfraktionen. Im Kolben lassen sich signifikante Unterschiede innerhalb der frühen Reifegruppe an Termin 2 und 4 und innerhalb der mittelfrühen Reifegruppe an Termin 3 und 4 feststellen. In der Gesamtpflanze unterscheiden sich die Stärkegehalte der frühen Reifegruppe an Termin 3 und der mittelfrühen Reifegruppe an Termin 2-4 signifikant voneinander. Zum Ende der Vegetationsperiode lassen sich keine signifikanten Unterschiede im Stärkegehalt des Kolbens und der Rest- bzw. Gesamtpflanze innerhalb der Reifegruppen nachweisen. Im Gegensatz dazu unterscheiden sich die Gehalte der Reifegruppen (Mittel der Sorten innerhalb einer Reifegruppe) zumeist signifikant voneinander. Abb. 1 stellt daher die signifikante Wechselwirkung 'Reifegruppe x Erntetermin' dar.

Tabelle 1.

Einfluss des Faktors 'Jahr' (year), 'Reifegruppe' (mat.), 'Sorte innerhalb einer Reifegruppe' (var. (mat.)), 'Erntetermin' (har.date) und 'Block' auf den Stärkegehalt von Kolben, Restpflanze und Gesamtpflanze. Varianzanalyse.

Table 1. Influence of the factors 'year', 'maturity group' (mat.), 'variety within maturity group' (var. (mat)), 'harvest date (har.date)' and 'block' on the contents of starch of ear, stover and whole-plant. Statistical analysis.

| ***STARCH*** | Num DF | | | F Value | | | Pr>F | | |
|---|---|---|---|---|---|---|---|---|---|
| Effect | ear | stover | whole-plant | ear | stover | whole-plant | Pr>F | stover | whole-plant |
| year | 2 | 2 | 2 | 251.85 | 91.48 | 246.15 | <.0001 | <.0001 | <.0001 |
| mat. | 2 | 2 | 2 | 118.82 | 8.62 | 268.64 | <.0001 | 0.0006 | <.0001 |
| var. (mat.) | 5 | 5 | 5 | 7.83 | 17.93 | 11.56 | <.0001 | <.0001 | <.0001 |
| har.date | 4 | 5 | 5 | 1571.26 | 643.72 | 3561.69 | <.0001 | <.0001 | <.0001 |
| block | 1 | 1 | 1 | 4.26 | 11.08 | 1.01 | 0.0474 | 0.0015 | 0.3208 |
| mat.*har.date | 8 | 10 | 10 | 9.90 | 17.09 | 37.68 | <.0001 | <.0001 | <.0001 |
| var. (mat.)*har.date | 20 | 25 | 25 | 3.21 | 2.16 | 4.18 | 0.0006 | 0.0144 | <.0001 |
| year*mat. | 4 | 4 | 4 | 2.20 | 4.90 | 5.40 | 0.0905 | 0.0019 | 0.0016 |
| year*var. (mat.) | 10 | 10 | 10 | 0.64 | 2.06 | 0.76 | 0.7706 | 0.0439 | 0.6679 |
| year*har.date | 8 | 10 | 10 | 28.43 | 40.44 | 34.30 | <.0001 | <.0001 | <.0001 |
| year*har.date*mat. | 16 | 20 | 20 | 5.82 | 2.41 | 2.78 | <.0001 | 0.0087 | 0.0031 |
| year*har.date*var.(mat.) | 40 | 50 | 50 | 1.07 | 1.19 | 1.01 | 0.416 | 0.2835 | 0.4871 |

Im Laufe der Vegetationsperiode steigen die Stärkegehalte im Kolben der frühen, mittelfrühen und mittelspäten Reifegruppe auf 539.1, 551.6 bzw. 566.7 g $kg^{-1}$ Trockenmasse (TM) an, wobei zum Ende der Vegetationsperiode signifikante Unterschiede zwischen der frühen und mittelspäten Reifegruppe nachgewiesen werden können (Abb. 1). Während die Sorten innerhalb der frühen Reifegruppe am 3. Erntetermin einen um 117 g $kg^{-1}$ TM höheren Stärkegehalt im Vergleich zu den mittelspäten Sorten aufweisen, beträgt der absolute Unterschied an Termin 6 nur noch 28 g $kg^{-1}$ TM.

Da die enzymatische Stärkebestimmung, die in dieser Untersuchung Anwendung fand, auch freie Glucose erfasst, ist davon auszugehen, dass es sich bei den Gehalten von bis zu 100 g $kg^{-1}$ TM in der Restpflanze (Abb. 1) um Vorstufen von Stärke handelt, die zum Kolben translociert werden. Aus diesem Grunde können auch die zu frühen Ernteterminen gemessenen Gehalte im Kolben (Termin 3-4) Anteile von Dextrinen enthalten, die noch nicht vollständig zu Stärke akkumuliert wurden. Phipps et al. (1984) dokumentieren in Blatt und Stängel nach der Blüte Stärkegehalte von bis zu 32 g $kg^{-1}$ TM.

Nach der Blüte (Termin 2) steigen die Stärkegehalte der Gesamtpflanze von 143 auf 349 g $kg^{-1}$ TM in der frühen Reifegruppe bzw. von 85 auf 300 g $kg^{-1}$ TM in der mittelspäten Reifegruppe (Abb. 1). Unter Berücksichtigung des TS-Gehaltes liegen die gemessenen Gehalte im Rahmen veröffentlichter Untersuchungen (u. a. Phipps et al., 1984; Andrieu et al., 1993). An Termin 3 findet sich die größte Differenz zwischen diesen beiden Reifegruppen (86 g $kg^{-1}$ TM), wobei die frühen Sorten stets einen signifikant höheren Stärkegehalt aufweisen als die mittelfrühen und die mittelspäten.

In Abhängigkeit der Reifegruppe werden nach der Blüte 206-221 g Stärke $kg^{-1}$ TM in der Gesamtpflanze akkumuliert. In Vergleichsstudien wird eine Stärkeakkumulation von 192 bzw. 190 g $kg^{-1}$ TM ab früher Milchreife beschrieben (Bal et al., 1997; Johnson et al., 1999).

Steigende Stärkegehalte während der Vegetationsperiode sind eng korreliert mit steigenden TS-Gehalten in der Gesamtpflanze (Coors et al., 1997; Ma et al., 2006) und sinkenden WLK- (Phipps et al., 1984) bzw. Gerüstsubstanzgehalten (Bal et al., 1997; Wiersma et al., 1993) in der Gesamtpflanze.

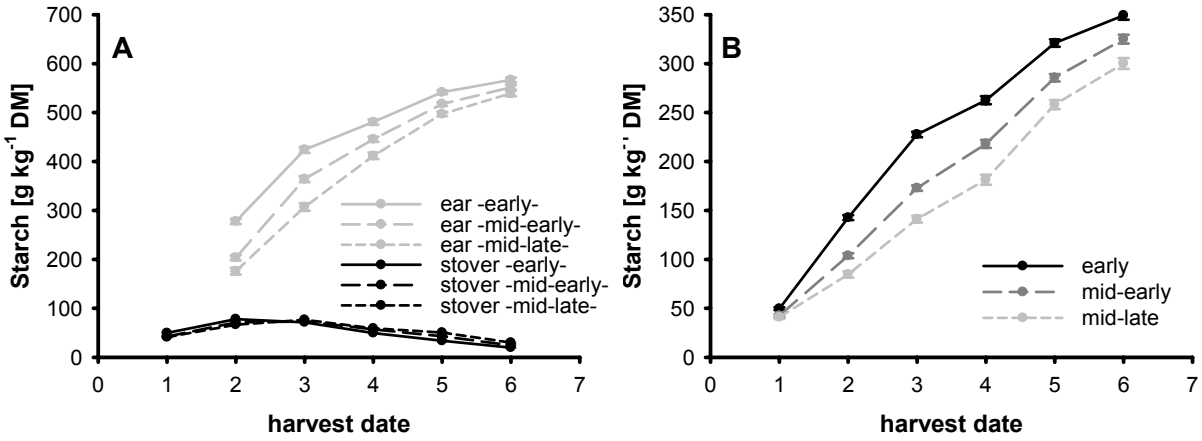


Abbildung 1.

Stärkegehalt [g $kg^{-1}$ TM] in Kolben und Restpflanze (A) sowie Gesamtpflanze (B) der frühen, mittelfrühen und mittelspäten Reifegruppe im Mittel der Jahre 2001-2003. Fehlerbalken: Standardfehler.

Figure 1. Starch content [g $kg^{-1}$ DM] in ear, stover (A) and whole-plant (B) of early, mid-early and mid-late maturity group, mean of three experimental years (2001-03). Bars representing standard error.

Zusammenfassend kann festgehalten werden, dass auch im Stärkegehalt keine signifikanten Sortenunterschiede innerhalb der Reifegruppe zum Ende der Vegetationsperiode in der Gesamtpflanze festgestellt werden können. Ähnlich zu den Gehalten an Zellwandbestandteilen und WLK unterscheiden sich die Reifegruppen in der Gesamtpflanze jedoch signifikant voneinander, so dass wiederum der Abreife der größte Einfluss auf die Stärkegehalte zugesprochen werden muss.

*5.3.1.2 Charakterisierung von Silomaisgenotypen anhand der Stärkegehalte*

*- umweltbedingte Variation*

Um eine abschließende Bewertung des Umwelteinflusses vornehmen zu können, wurden die Stärkegehalte, die nach der Blüte in der Gesamtpflanze ermittelt wurden, modellmäßig betrachtet (Abb. 2). Modellkalibrationen wurden für jede Sorte einzeln aufgestellt, aus Übersichtsgründen werden hier jedoch nur die Verläufe der ausgewählten Sorten Oldham (frühe Reifegruppe, normalabreifend) und Fuego (mittelfrühe Reifegruppe, stay green Typ) vorgestellt.

Wie bereits bei den vorangegangenen Modellkalkulationen für andere Qualitätsparameter beschrieben (Kapitel 3, 4), bewirken kühle Temperaturen im Versuchsjahr 2001 eine verzögerte Abreife der Maispflanzen. Die modellierten Stärkegehalte der Gesamtpflanze steigen

im Jahr 2001 zu einem späteren Zeitpunkt und zugleich langsamer an als die Gehalte in den Jahren 2002 und 2003. Auch hier zeigt sich kein Effekt der geringeren Niederschläge des Versuchsjahres 2003, obwohl niedrigere Stärkegehalte bei Trockenstress dokumentiert sind (Maranville und Paulsen, 1970). Wenn die Photosyntheseleistung aufgrund des fehlenden Wassers reduziert ist, wird der geringere Stärkegehalt u. a. auf das verringerte Assimilatangebot zurückgeführt (Ober et al., 1991). Durch das späte Auftreten (ca. 14 Tage nach der Blüte) des Trockenstresses im Jahre 2003 (Kap. 3, Abb. 1) wird die Stärkeakkumulation nicht verringert, wohingegen die Temperatur die Entwicklung von Maispflanzen und somit auch die Stärkegehalte in erheblicherem Maße beeinflusst (u. a. Struik, 1983).

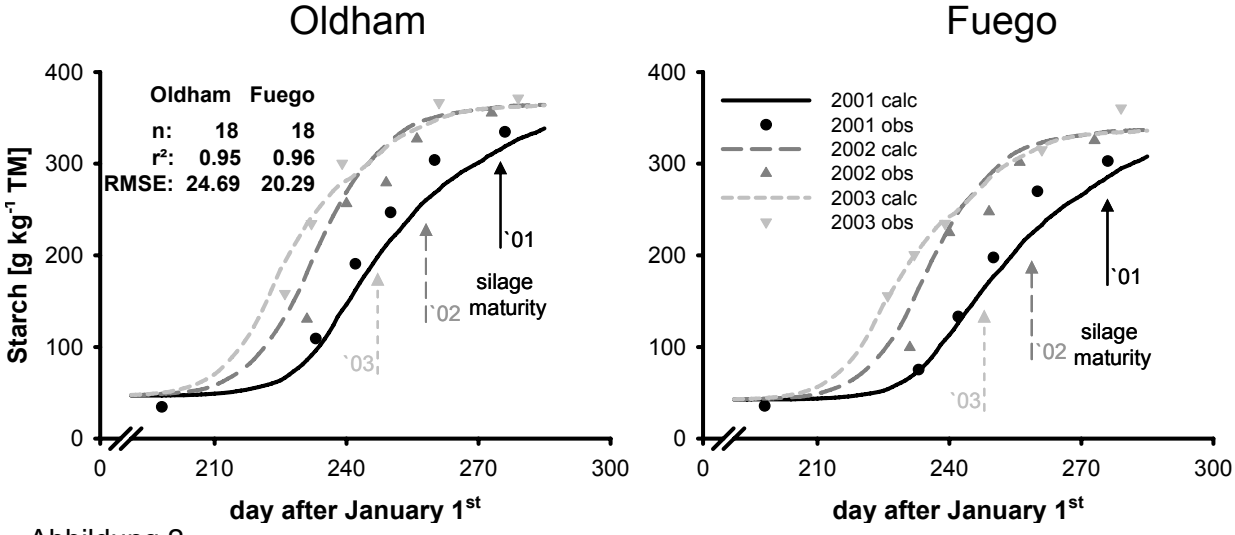


Abbildung 2.

Berechnete (Linien) und gemessene (Symbole) Stärkegehalte in der Gesamtpflanze der Sorten Oldham und Fuego in den Jahren 2001-03. Pfeile markieren Siloreife (~320 g $kg^{-1}$ TM).

Figure 2. Calculated (lines) and observed (symbols) contents of starch in the whole-crop of the selected varieties Oldham and Fuego for experimental years 2001-2003. Arrows indicating silage maturity (~320 g DM kg).

Im Bereich der Siloreife betragen die Unterschiede zwischen den Jahren 2002 und 2003 bis zu 65.6 g Stärke $kg^{-1}$ TM (Oldham), wohingegen die beiden Sorten zur Siloreife maximal um 39.7 g Stärke $kg^{-1}$ TM (2003) variieren. Die Vergleiche zwischen den berechneten und gemessenen Stärkegehalten verdeutlichen eine gute Modellanpassung. Diese wird durch Bestimmtheitsmaße von ~ 0.95 und Schätzfehlern (RMSE) unter 25 g $kg^{-1}$ TM belegt.

### *5.3.2 Charakterisierung von Silomaisgenotypen anhand der phänologischen Entwicklung und der Abreife*

Die in dieser Arbeit untersuchten Silomaisgenotypen wurden dahingehend ausgewählt, dass sie das Sortenspektrum von Silomais abdecken. Neben unterschiedlichen Inhaltsstoffzusammensetzungen, wurden verschiedene Reifegruppen (früh, mittelfrüh, mittelspät) und Abreifetypen (normal, stay green, dry down) berücksichtigt (Tab. 2).

Die bereits vorgestellten Untersuchungen bezüglich der Gehalte an Zellwandbestandteilen bzw. der Zucker- und Stärke-Gehalte zeigen, dass die ermittelten Reifegruppenunterschiede nur bedingt auf genetische Variabilität zurückzuführen sind. Die höheren Gerüstsubstanzgehalte der mittelspäten Sorten in der Gesamtpflanze, kombiniert mit höheren WLK- bzw. niedrigeren Stärkegehalten und die entsprechend gegenläufigen Gehalte der frühen Sorten weisen auf abreifebedingte Unterschiede bezüglich der Gehalte an Qualitätsparametern hin.

Tabelle 2.
Sortencharakteristika des geprüften Maissortiments.

Table 2. Characteristics of the tested maize cultivars.

| Sorte | Siloreifezahl (SRZ) | Kornreifezahl (KRZ) | Reifegruppe | Abreifetyp[#] | Korntyp[##] |
|---|---|---|---|---|---|
| Arsenal | 210 | 210 | früh | normal | zw |
| Oldham | 220 | - | früh | normal | zw |
| Symphony | 220 | 210 | früh | staygreen* | zw |
| Probat | 230 | 240 | mittelfrüh | dry down* | (za) zw |
| Attribut | 240 | 250 | mittelfrüh | dry down* | ha |
| Fuego | 250 | 220 | mittelfrüh | stay green ** | zw |
| Clarica | 270 | 280 | mittelspät | dry down* | za |
| Benicia | 280 | 250 | mittelspät | stay green ** | (ha) zw |

#: *,**: Ausprägung der entsprechenden Merkmals

##: ha: Hartmais; zw: Zwischenform; za: Zahnmais

#### *5.3.2.1 Charakterisierung von Silomaisgenotypen anhand des Kolbenanteils*

Die Abreife von Silomais in der generativen Phase ist durch Verschiebungen in der morphologischen Zusammensetzung, vor allem durch den steigenden Kolbenanteil an der Gesamtpflanze, charakterisiert. Bei erfolgreicher Befruchtung der Körner steigt der Kolbenanteil der

Maispflanzen durch die dort stattfindende Stärkeakkumulation im weiteren Vegetationsverlauf an (Phipps et al.,1984; Coors et al., 1997), wohingegen die Anteile der Stängel und Blätter abnehmen und im Bereich der Siloreife Werte von 30 bzw. 15% erreicht werden (Phipps et al., 1984; Verbic et al., 1995). Die Kolben nehmen zur Siloreife einen Gewichtsanteil von bis zu 55% ein (Phipps et al., 1984; Verbic et al., 1995).

Die varianzanalytische Verrechnung der Kolbenanteile zeigt keine signifikanten Sortenunterschiede innerhalb der Reifegruppen zu entsprechenden Ernteterminen (Tab. 3). Frühe Sorten weisen zu jedem Zeitpunkt in der Vegetationsperiode einen höheren Kolbenanteil auf als mittelfrühe und diese wiederum höhere als mittelspäte (Abb. 3). Größte Differenzen zwischen den Reifegruppen finden sich am Erntetermin 3 (bis zu 10% höherer Kolbenanteil der frühen Reifegruppe), wohingegen sich zum Ende der Vegetationsperiode die Kolbenanteile annähern und zwischen 53% und 61% variieren (Abb. 3A).

Tabelle 3.
Einfluss des Faktors 'Jahr' (year), 'Reifegruppe' (mat.), 'Sorte innerhalb einer Reifegruppe' (var. (mat)), 'Erntetermin' (har. date) und 'Block' auf den Kolbenanteil. Varianzanalyse.

Table 3. Influence of the factors 'year', 'maturity group' (mat.), 'variety (var.) within maturity group' 'harvest date (har.date)' and 'block' on the ear contents. Statistical analysis.

***ear content***

| Effect | Num DF | F Value | Pr>F |
|---|---|---|---|
| year | 2 | 63.03 | <.0001 |
| mat. | 2 | 412.86 | <.0001 |
| var. (mat.) | 5 | 22.25 | <.0001 |
| har. date | 4 | 1287.50 | <.0001 |
| block | 1 | 2.20 | 0.1473 |
| mat.*har. date | 8 | 10.69 | <.0001 |
| var. (mat.)*har. date | 20 | 1.32 | 0.2163 |
| year*mat. | 4 | 12.63 | <.0001 |
| year*var. (mat.) | 10 | 1.42 | 0.2137 |
| year*har. date | 8 | 14.29 | <.0001 |
| year*har.d ate*mat. | 16 | 1.16 | 0.3337 |
| year*har. date*var.(mat.) | 40 | 1.12 | 0.3564 |

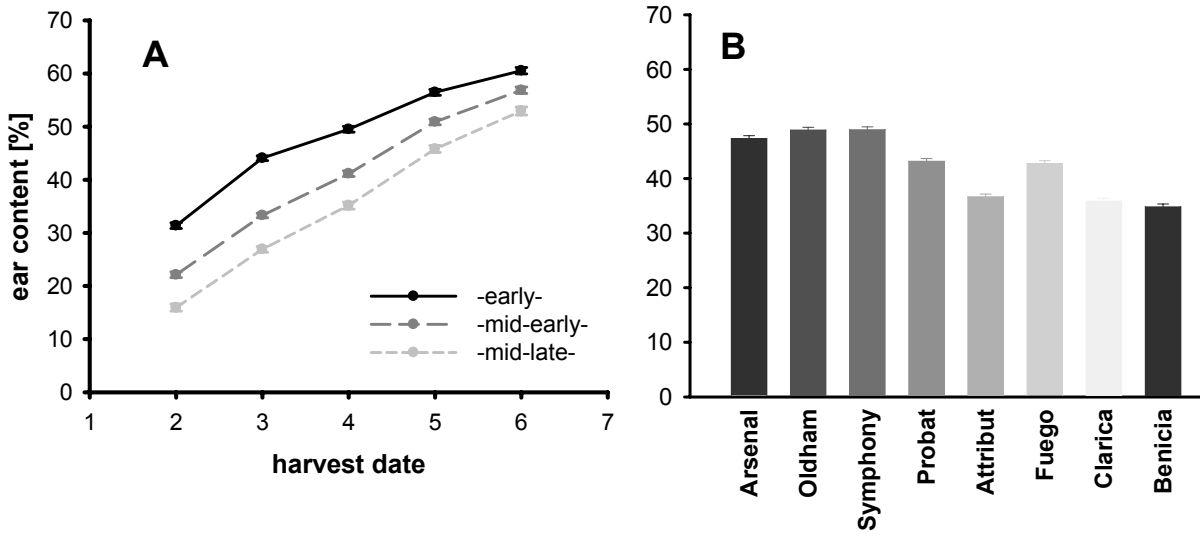


Abbildung 3.

A. Kolbenanteil [%] der frühen, mittelfrühen und mittelspäten Reifegruppe im Mittel der Jahre 2001-2003. Fehlerbalken: Standardfehler. B. Kolbenanteil [%] der einzelnen Sorten im Mittel über die Jahre und Erntetermine.

Figure 3 A. Ear content [%] of early, mid-early and mid-late maturity group, mean of three experimental years (2001-03). Bars representing standard error. 3 B. Ear content [%] of silage maize hybrids in the mean of experimental years and harvest dates.

Verbic et al. (1995) dokumentieren Sortenunterschiede im Bereich der Siloreife (319 +/- 3.3 g TM $kg^{-1}$ fresh matter) bezüglich der morphologischen Zusammensetzung; dieses kann in dieser Untersuchung innerhalb der Reifegruppe zu entsprechenden Ernteterminen nicht belegt werden. Im Mittel der Jahre und der Erntetermine weist jedoch die Sorte 'Attribut' innerhalb der mittelfrühen Reifegruppe einen signifikant niedrigeren Kolbenanteil auf (Abb. 3B). Philippeau und Michalet-Doreau (1997) machen Heterosis-Effekte für den stets höheren Kolbenanteil von Zahnmais in ihrer Studie verantwortlich.

*5.3.2.2 Charakterisierung von Silomaisgenotypen anhand des TS-Gehaltes*

Die voranschreitende Abreife der Maispflanzen ist neben steigenden Kolbenanteilen durch den zunehmenden TS-Gehalt in der Gesamtpflanze charakterisiert (Phipps und Weller, 1979; Bal et al., 1997; Ma et al., 2006).

Signifikante Sortenunterschiede innerhalb der Reifegruppe bezüglich des TS-Gehaltes in der Gesamtpflanze können nur zu frühen Entwicklungsstadien abgesichert werden (Tab. 4). Im Kolben-TS-Gehalt zeigen sich signifikante Sortenunterschiede innerhalb der frühen und

mittelfrühen Reifegruppe bis zum ernterelevanten Erntetermin 5, die sich auf ~40 g TM $kg^{-1}$ belaufen. Die Sorten Oldham (früh) und Fuego (mittelfrüh) weisen in ihrer Reifegruppe niedrigere bzw. höhere Kolben-TS-Gehalte auf. Im geprüften Sortiment ist nach der Blüte (Termin 2) sowohl im Kolben als auch in der Gesamtpflanze ein nahezu linear steigender TS-Gehalt zu verzeichnen (Abb. 4). Zum Ende der Vegetationsperiode weisen die frühen Sorten (393.9 g TM $kg^{-1}$) einen signifikant höheren TS-Gehalt in der Gesamtpflanze im Vergleich zu den mittelfrühen (374.4 g TM $kg^{-1}$) und mittelspäten (323.5 g TM $kg^{-1}$) Sorten auf.

Tabelle 4.
Einfluss des Faktors 'Jahr' (year), 'Reifegruppe' (mat.), 'Sorte innerhalb einer Reifegruppe' (var. (mat)), 'Erntetermin' (har. date) und 'Block' auf den TS-Gehalt. Varianzanalyse.

Table 4. Influence of the factors 'year', 'maturity group' (mat.), 'variety (var.) within maturity group' 'harvest date (har.date)' and 'block' on the DM content. Statistical analysis.

| | Num DF | | F Value | | Pr>F | |
|---|---|---|---|---|---|---|
| Effect | whole-plant | ear | whole-plant | ear | whole-plant | ear |
| year | 2 | 2 | 732.33 | 424.57 | <.0001 | <.0001 |
| mat. | 2 | 2 | 88.12 | 162.58 | <.0001 | <.0001 |
| var. (mat.) | 5 | 5 | 2.19 | 22.05 | 0.0731 | <.0001 |
| har. date | 5 | 4 | 2469.61 | 2897.52 | <.0001 | <.0001 |
| block | 1 | 1 | 8.75 | 17.98 | 0.0049 | 0.0002 |
| mat.*har. date | 10 | 8 | 8.50 | 6.22 | <.0001 | <.0001 |
| var. (mat.)*har. date | 25 | 20 | 2.34 | 2.76 | 0.0114 | 0.0017 |
| year*mat. | 4 | 4 | 9.88 | 8.82 | <.0001 | 0.0001 |
| year*var. (mat.) | 10 | 10 | 2.49 | 2.28 | 0.0193 | 0.0425 |
| year*har. date | 10 | 8 | 99.64 | 58.32 | <.0001 | <.0001 |
| year*har.d ate*mat. | 20 | 16 | 1.44 | 2.26 | 0.1734 | 0.0139 |
| year*har. date*var.(mat.) | 50 | 40 | 0.88 | 1.12 | 0.668 | 0.3424 |

TS-Gehalte in der Gesamtpflanze zwischen 300 und 350 g TM $kg^{-1}$ werden als optimal angesehen, um sowohl einen optimalen Silierprozess mit geringen Nährstoff- und TM-Verlusten (Allen et al., 2003), als auch eine hinreichende TM-Aufnahme der Wiederkäuer (Böhm et al., 1983) sicherzustellen. Im Mittel der Jahre haben alle Sorten den optimalen TS-Gehalt in der Gesamtpflanze erreicht und teilweise überschritten (Abb. 4). Während die frühen Sorten durchschnittlich 3.9 g TM $kg^{-1}$ $d^{-1}$ akkumulieren, liegt die Akkumulationsrate für die mittelspäten bei 2.8 g TM $kg^{-1}$ $d^{-1}$. Die Reifeklassifizierung der untersuchten Sorten kann für die Nutzungsrichtung Silomais für die Extrema der untersuchten Sorten zum letzten Erntetermin bestätigt werden. Arsenal (SRZ 210/ KRZ 210) hat zum Ende der Vegetationsperiode einen um etwa 80 g $kg^{-1}$ höheren TS-Gehalt in der Gesamtpflanze als Benicia (SRZ 280/ KRZ

250), welches in etwa der Abstufung in der Reifeklassifizierung (Anonymus, 2006c) entspricht. Die Unterschiede im Kolben-TS-Gehalt fallen bei einem Unterschied von 70 g TM $kg^{-1}$ dagegen höher aus als die Kornreifezahl erwarten läßt.

Die festgestellten Sortenunterschiede innerhalb der frühen und mittelfrühen Reifegruppe wirken sich nicht signifikant auf die morphologische Zusammensetzung bzw. auf den TS-Gehalt der Gesamtpflanze aus. Die Differenzierung zwischen den Reifegruppen ist in der Gesamtpflanze einzig durch die unterschiedlich schnelle Entwicklung bedingt. Der Klassifizierung anhand der TS-Gehalte der Gesamtpflanze kann weitestgehend entsprochen werden.

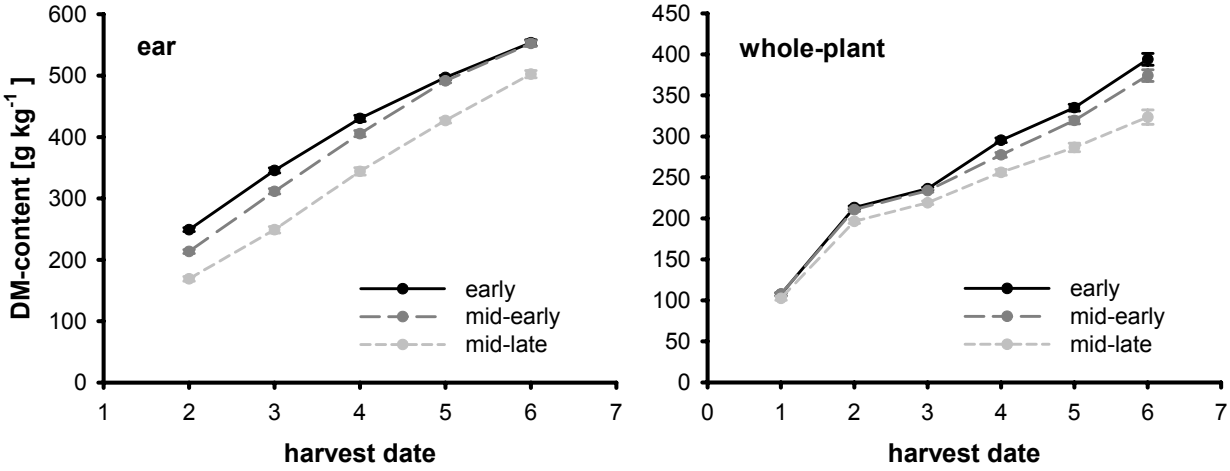


Abbildung 4.
Kolben und Gesamtpflanzen TS-Gehalt [g $kg^{-1}$] der frühen, mittelfrühen und mittelspäten Reifegruppe im Mittel über die Jahre 2001-2003. Fehlerbalken: Standardfehler.

Figure 4. Ear and whole-plant dry matter content [g $kg^{-1}$] of early, mid-early and mid-late maturity group, mean of three experimental years (2001-03). Bars representing standard error.

*5.3.3 Abschließende Charakterisierung von Silomaisgenotypen*

Trotz der Berücksichtigung unterschiedlichster Abreifetypen in den Reifegruppen können die in frühen Stadien der Entwicklung aufgezeigten Sortenunterschiede in den Gehalten an Qualitätsparametern (NDF, ADF, Hemicellulose, Cellulose, Lignin, Stärke, WLK) in Kolben, Rest- und v. a. der Gesamtpflanze zum Ende der Vegetationsperiode nur bedingt abgesichert werden. Außer im WLK-Gehalt unterscheiden sich die Gesamtpflanzen der Sorten innerhalb einer Reifegruppe zum Ende der Vegetationsperiode nicht signifikant voneinander. Zur weiteren Bewertung der Sorten- bzw. Reifegruppenunterschiede muss die Versuchsanlage Berücksichtigung finden. Die Erntetermine in dieser Studie richteten sich nach dem Entwicklungsstadium der mittelfrühen Referenzsorte Probat, d.h. wenn diese Sorte das

BBCH-Stadium 32, bzw. einen TS-Gehalt im Kolben von 200, 300, 400, 500 und 550 g $kg^{-1}$ Frischmaterial erreichte, wurden sämtliche Sorten beprobt. Diese Vorgehensweise führte bei gleichen Aussaatterminen dazu, dass frühe Sorten zu den entsprechenden Ernteterminen im Allgemeinen weiter abgereift waren als die mittelfrühen und insbesondere im Vergleich zu den mittelspäten ein höheres physiologisches Alter aufwiesen. Dieses wird durch die Unterschiede in den TS-Gehalten (Abb. 4) belegt.

Zur Bestimmung des physiologischen Alters werden in Abb. 5 die Parameter Gesamtpflanzen-TS-Gehalt bzw. Kolbenanteil herangezogen und die Abhängigkeiten der Stärke-, NDF- und WLK- Gehalte dargestellt. Die Reifegruppen zeigen keine Unterschiede in den entsprechenden Beziehungen. Die nach der Blüte ermittelten Gehalte an Stärke, WLK und NDF sind für alle Reifegruppen im Mittel über die Versuchsjahre gleichermaßen mit dem TS-Gehalt korreliert. Ein positiv linearer Zusammenhang besteht zwischen den steigenden TS-Gehalten (bis 400 g TM $kg^{-1}$) und den Stärkegehalten in der Gesamtpflanze (Steigung: 1.31), wohingegen die Gehalte an WLK (-1.24) und NDF (-0.66) bei steigendem TS-Gehalt linear sinken. Hohe Bestimmtheitsmaße (>0.85) und Fehler unter 26 g $kg^{-1}$ TM bestätigen die enge Beziehung zwischen den betrachteten Parametern. Vor allem der Kolbenanteil und die Stärkegehalte sind eng miteinander korreliert ($R^2$= 0.99; S.E.= 9.81 g $kg^{-1}$ TM).

Ein linearer Zusammenhang zwischen den TS- und Stärke-Gehalten wurde ebenfalls bei Wilkinson (1978) und van Waes et al. (1997) beschrieben. Givens und Deaville (2001) dokumentierten in Abhängigkeit des TS-Gehaltes nicht-lineare Zusammenhänge zwischen steigenden Stärke- und fallenden NDF-Gehalten in älteren (1970) und neueren (1980-90) Silomaissorten. Für das neuere Sortiment konnte jedoch der nicht-lineare Zusammenhang zwischen Stärke und TS-Gehalt nicht signifikant belegt werden. Steigende NDF-Gehalte nach der Siloreife in der Gesamtpflanze (Wiersma et al., 1993; Bal et al., 1997; Givens und Deaville, 2001) konnten in dieser Studie im Mittel über die Jahre ebenso wie konstant bleibende Stärke-Gehalte (Bal et al., 1997) nicht nachgewiesen werden.

Zusammenfassend kann also festgehalten werden, dass die ermittelten Unterschiede zwischen den Reifegruppen in der Gesamtpflanze bezüglich des WLK-, Stärke-, NDF-, ADF, Hemicellulose- und Cellulose- Gehalts nahezu ausschließlich auf Unterschiede in der Abreife zurückzuführen sind. Vor dem Hintergrund der alternativen Nutzung der Maissilage zur Biogasproduktion werden momentan in Deutschland frühere Erntetermine diskutiert (Amon

et al., 2004), sodass in früheren Entwicklungsstadien absicherbare Sortenunterschiede von zukünftiger Bedeutung sein könnten.

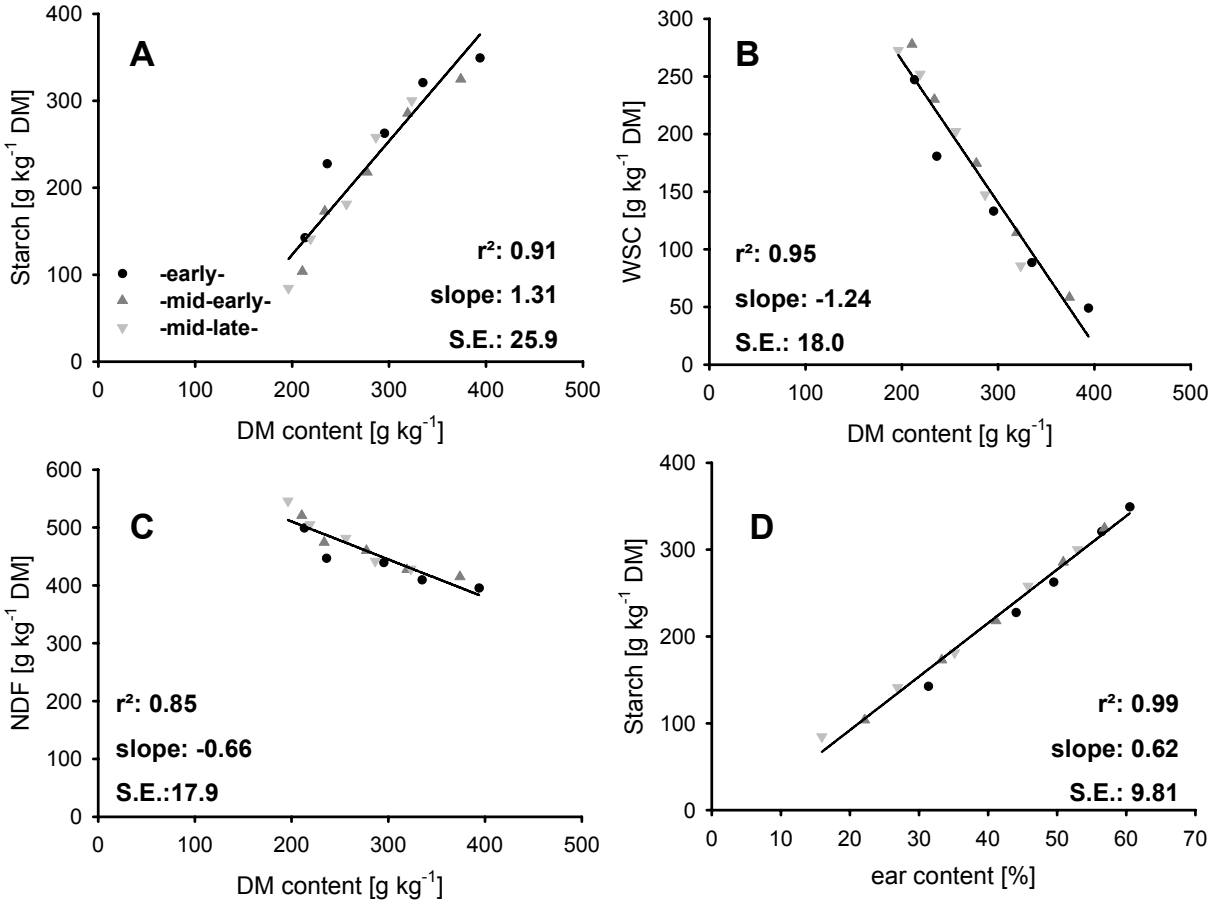


Abbildung 5.

Gehalt an Stärke, wasserlöslichen Kohlenhydraten (WSC) und NDF der Gesamtpflanze in Abhängigkeit des TS-Gehaltes (DM; A-C), bzw. der Stärkegehalt in Abhängigkeit des Kolbenanteils (D) der frühen, mittelfrühen und mittelspäten Reifegruppe. Erntetermine dargestellt im Mittel der Jahre 2001-2003.

Figure 5. Starch, WSC and NDF content of the whole crop in relation to their DM content (A-C) and starch content in relation to the ear content (D) of the whole crop of early, mid-early and mid-late maturity group in the mean of 2001-2003.

*5.4 Futterwertbestimmung von Silomais - methodische Aspekte*

Die Nah-Infrarot-Reflexions-Spektroskopie (NIRS) bietet die Möglichkeit, eine große Probenzahl ohne Aufwand an Reagenzien zeit- und kosteneffizient zu analysieren. Neben den Standardmethoden zur Bestimmung der Futterqualität von Silomais, wie dem Stärkegehalt, dem Rohfaser-, dem Rohprotein- sowie dem Energiegehalt, ist die NIRS-Methode zur Schätzung weiterer den Futterwert direkt bestimmende Parameter etabliert (Murray et al.,

1993; Stuth et al., 2003). Untersuchungen zur NIRS-Bestimmung der Gasbildung in Anlehnung an den Hohenheimer-Futterwerttest (Herrero et al., 1997; Lovett et al., 2004) lassen jedoch ebenso wie erste Ergebnisse zur Schätzung der Methanausbeute weiteren Forschungsbedarf erkennen (Krützfeld et al., 2005).

Für die Bestimmung der Gerüstsubstanzen und der Nicht-Struktur-Kohlenhydrate wurden im Rahmen der vorliegenden Arbeit separate Kalibrationen für die Pflanzenfraktionen Kolben und Restpflanze auf Basis der beschriebenen naßchemischen Analysen aufgestellt. Kalibrations- und Validationssubsets umfassten die gesamte Probenpopulation. Die Güte der NIRS-Schätzgleichungen, wie sie in Kapitel 3 und 4 beschrieben ist, zeigt hohe Bestimmtheitsmaße und geringe Fehler, so dass auch hier die NIRS einmal mehr ihre Vorzüglichkeit beweisen konnte. Untersuchungen zur Schätzgenauigkeit von NIRS bei der Bestimmung von Inhaltsstoffen im heterogenen Gesamtpflanzenmaterial wurden nicht vorgenommen. Volkers et al. (2003) belegen die höhere Genauigkeit der NIRS-Schätzgleichungen bezüglich der Bestimmung von Qualitätsparametern in homogenem Pflanzenmaterial von Silomais.

Die Inhomogenität der Gesamtpflanze, die sich bei Mais als faserdominierte Restpflanze und stärkedominiertem Kolben darstellt (Abb. 3), scheint ebenfalls verantwortlich für die Ungenauigkeit der NIRS zur Schätzung der Gasbildung (Lovett et al., 2004). Jedoch verbessert eine Fraktionierung in Kolben und Restpflanze die Schätzgenauigkeit nur bedingt (Kapitel 2). Das in dieser Untersuchung vorliegende Probenmaterial beinhaltet Restpflanzen und Kolben mit großem Abreifegradienten und unterschiedlicher Inhaltsstoffzusammensetzung. Da Kohlenhydratfraktionen zwar in unterschiedlichen Raten, jedoch zeitgleich fermentiert werden (Cone et al., 1997), kann die Gasbildung momentan nicht exakt über die Gehalte der entsprechenden chemischen Inhaltsstoffe geschätzt werden.

Gerade vor dem Hintergrund großer Probenpopulationen bietet die NIRS-Methode große Vorteile bezüglich der chemischen Analyse von Inhaltsstoffen. Bei einer balancierten Abdeckung der Variabilität innerhalb der Probenpopulation durch zu analysierende Subsets (Smith und Kearney, 2000) kann die NIRS-Methode routinemäßig eingesetzt werden, um die Zusammensetzung chemischer Inhaltsstoffe sicher zu bestimmen.

Vor allem in der Pflanzenzüchtung und zur Klassifizierung einzelner Genotypen ist die Fraktionierung der Maisgesamtpflanze in vegetative und generative Pflanzenteile vor dem Hintergrund verbesserter Schätzgenauigkeiten sinnvoll. Der Vorteil der größeren und

detaillierteren Datengrundlage zur Evaluierung des Probenmaterials übertrifft den technischen Mehraufwand.

*5.5 Simulationsmodelle zur Bestimmung der umweltbedingten Variabilität*

Umweltfaktoren, wie Temperatur, Einstrahlung und Niederschlag, beeinflussen in erheblichem Maße direkt oder indirekt die Ertrags- und Qualitätsentwicklung von Silomais. Vor allem in sensitiven Phasen der Entwicklung werden Ertrags- und Qualitätsentwicklung von Silomais durch unterschiedliche Witterungsbedingungen beeinflusst (u.a. Struik 1983). Während Untersuchungen zu Auswirkungen von Witterungsbedingungen auf unterschiedliche physiologische Prozesse vielfach vorliegen, fokussieren etablierte Simulationsmodelle vor allem auf Ertragseigenschaften von Silomais (vgl. Herrmann et al., 2005). Dabei bieten computergesteuerte Simulationsmodelle die Möglichkeit, neben den Auswirkungen genetischer Voraussetzungen und Managemententscheidungen, Einflüsse der Umweltbedingungen auf die Ertrags- und Qualitätsentwicklung von Nutzpflanzen zu beschreiben.

Die große Bedeutung der Witterungsbedingungen wird durch den signifikanten Einfluss des Faktors 'Jahr' als Haupteffekt oder in Wechselwirkung auf alle in dieser Studie betrachteten Qualitätsparameter (Kapitel 3, 4; Tab. 1) belegt. Mit Hilfe der witterungsgesteuerten, dynamischen Modelle FOPROQ und FONSCH, die ursprünglich für Grünlandaufwüchse entwickelt wurden, konnte eine Quantifizierung des Einflusses von Tagesdurchschnittstemperatur, Einstrahlung und pflanzenverfügbarem Bodenwasser auf die Gehalte an Gerüstsubstanzen (Kapitel 3) bzw. auf die WLK-Gehalte (Kapitel 4) und Stärke (Abb. 1) vorgenommen werden. Insbesondere der Einfluss der Temperatur spiegelte sich durch verzögerte Abreifen im Versuchsjahr 2001 wider. Auftretender Trockenstress beeinflusste die Qualitätsentwicklung nicht substantiell. Die Gehalte an qualitätsbestimmenden Parametern unterschieden sich zur Siloreife, also bei gleichem physiologischen Alter, nicht erheblich voneinander (Kapitel 3 und 4). Während sich die Unterschiede zwischen den Sorten auf etwa 35 g WLK, 27 g NDF und 66 g Stärke $kg^{-1}$ TM beliefen, wurden im Bereich der Siloreife Differenzen zwischen den Jahren von 100 g WLK, 43 g NDF und 40 g Stärke $kg^{-1}$ TM festgestellt. Epinat- Le Signor et al. (2001) belegten, dass 80% der Variation des Maisertrages in Nord-Frankreich auf Umwelteinflüsse zurückgeführt werden konnte, während der genotypbedingte Effekt (6%) geringere Auswirkungen zeigte als der Effekt der Genotyp x Umwelt Interaktion (11%). In einer Simu-

lationsstudie von Andresen et al. (2001) wird der Temperatur neben der Wasserverfügbarkeit eine große Bedeutung auf den Maisertrag, vor allem in nördlicheren Gebieten der USA, zugeschrieben. Eine Verbesserung der Prognosegüte könnte durch differenzierte Betrachtung der Temperaturauswirkungen in unterschiedlichen Entwicklungsstadien erreicht werden, wie sie u. a. von Stewart et al. (1998) beschrieben werden.

Die Sensitivität der betrachten Qualitätsparameter auf herrschende Umweltbedingungen wurde durch Simulationsstudien (Kapitel 3 und 4) belegt. Umweltbedingungen beeinflussen in vergleichsweise geringem Maße die Gerüstsubstanzgehalte, wohingegen die TS- und Stärkegehalte (Kruse et al., 2006) und insbesondere die Gehalte an WLK erheblichen umweltbedingten Schwankungen unterworfen sind. In einer Simulationsstudie über 25 Jahre konnten Herrmann et al. (2005) die relativ gute Übereinstimmung zwischen geschätzten und gemessenen Gehalten im siloreifen Mais bezüglich Stärke- und TS-Gehalt belegen. Außerdem wurde dokumentiert, dass in über 60% der betrachteten Jahre (1979-2003) die Siloreife (~320 g TS $kg^{-1}$) aufgrund ungünstiger Witterungsbedingungen von frühen und mittelfrühen Silomaissorten nicht erreicht wurde; dieses Ergebnis bezieht sich auf eine Schnitthöhe von <5 cm. Die Notwendigkeit, die Zusammensetzung chemischer, qualitätsbestimmender Parameter nicht nur im Bereich der Siloreife zu beschreiben, sondern auch die Qualitätsentwicklung zu berücksichtigen, wird durch die oftmals verfrühte Ernte von Silomais verdeutlicht. Die Auswirkungen der Temperatur und der Einstrahlung auf die Photosynthese-Leistung und Translokation von Assimilaten auch in späteren Entwicklungsstadien stellen den bedeutenden Faktor in der Qualitätsentwicklung von Silomais unter norddeutschen Verhältnissen dar, die vornehmlich nicht durch Wasser- bzw. Nährstoff-Mangel charakterisiert sind.

Im Projekt 'Regionale Erntezeitprognose von Silomais' wurden reine Temperatur-Indices ('growing-degree-days') mit unterschiedlichen Basistemperaturen (8°C, 6°C nach AGPM und DMK) gegenüber FOPROQ getestet, um eine sichere Erntezeitvorhersage von Silomais zu entwickeln. Mit dem modifizierten Modell MAISPROG konnte durch die Implementierung zusätzlicher Klimadaten, wie der Einstrahlung und dem pflanzenverfügbaren Bodenwasser, eine Steigerung der Schätzgenauigkeit im Vergleich zu den Temperatursummen-Ansätzen erreicht werden (Rath et al., 2005). MAISPROG ist bereits in der Praxis etabliert und bietet den Landwirten die Möglichkeit, die optimale Erntezeitspanne von Silomais frühzeitig zu erkennen. Dem Versuchswesen wird die Möglichkeit eröffnet, Effizienzsteigerungen bei der

logistischen Planung der Erntefolge verschiedener Versuchsstandorte zu erzielen und eine treffendere Sortenbeurteilung vornehmen zu können (Rath et al., 2005). Die Vorhersage von Inhaltsstoffgehalten auf Basis von Wetterdaten mittels adäquater Modelle, die hier ihre Eignung bewiesen haben, könnte zusätzliche Informationen über die Qualitätsentwicklung von Silomais liefern.

Bei Erweiterung der vorliegenden Datenbasis durch Einbeziehung verschiedener Umwelten und bei erfolgreicher Validation der Modellparameter könnten Erntezeitpunktprognosen durch Aussagen über die Qualitätsentwicklung unterstützt werden.

### *5.6 Abschließende Bewertung und Implikationen für die Züchtung*

Die Futterqualität von Silomais kann nicht allein durch die Zusammensetzung von Inhaltsstoffen definiert werden, da die Abbaubarkeit der einzelnen Qualitätsparameter in erheblichem Maße den Futterwert determiniert (Jung und Deetz, 1993; Jensen et al., 2005). In der vorliegenden Studie sollte die Gasbildung in Anlehnung an den Hohenheimer-Futterwert-Test (Menke und Steingass, 1988) zur Bewertung des energetischen Futterwertes herangezogen werden, um vor dem Hintergrund unterschiedlicher ruminaler Abbaubarkeiten verschiedener Inhaltsstofffraktionen die untersuchten Silomaissorten weitergehend zu charakterisieren. Aufgrund der beschriebenen Probleme hinsichtlich der Bestimmung der Gasbildung mittels NIRS (Kapitel 2) konnte jedoch keine umfassende Analyse des Probenmaterials erfolgen. Einjährige Ergebnisse (Versuchsjahr 2001) weisen darauf hin, dass Sortenunterschiede hinsichtlich der Gasbildung v. a. bei Betrachtung der Restpflanze zu erwarten sind (Beckmann, 2003). Da in der Studie von Beckmann (2003) keine Gruppierung der Sorten innerhalb einzelner Reifegruppen vorgenommen wurde, können allerdings keine Schlüsse bezüglich Sortenunterschieden, die nicht in Abhängigkeit des Abreifegrades stehen, gezogen werden.

In der vorliegenden Studie wurde die Möglichkeit aufgezeigt, verschiedene Silomaisgenotypen anhand futterwertbestimmender Parameter, insbesondere der Kohlenhydratfraktionen zu charakterisieren. Die Ergebnisse weisen auf marginale genotypbedingte Unterschiede in den Gehalten von Qualitätsparametern zum Ende der Vegetationsperiode hin, absicherbare Unterschiede sind nahezu ausschließlich auf unterschiedliche Abreifen der Genotypen zurückzuführen. Eine abschließende Differenzierung der Silomaisgenotypen hinsichtlich des Futterwertes ist aufgrund der nicht ermittelten Abbaukinetiken einzelner Inhaltsstoffe nicht

möglich. Sorteneffekte auf die Abbaubarkeiten einzelner Inhaltsstoffe einerseits (Barrière et al., 2003a, b) und auf tierische Leistungsparameter andererseits (Johnson et al., 2002) sind dokumentiert. Weitere Forschungsaktivitäten müssen klären, ob diese Differenzierungen von Silomaissorten auch bei systematischer Analyse verschiedener Abreifetypen innerhalb einer Reifegruppe bestätigt und durch sortenspezifische Parameter, wie z. B. der physikalischen Struktur, erklärt werden können. Ausschließlich durch die chemische Analyse von Qualitätsparametern kann keine genotypbedingte Differenzierung des Silomaissortenspektrums festgestellt werden. Die momentan gängige Beschreibung der Genotypen anhand der Gehalte von Rohnährstoffen ist vor dem Hintergrund der Ergebnisse der vorgestellten Studie zur näheren Charakterisierung von Silomaissorten unzureichend.

Zur züchterischen Steigerung der Futterqualität von Silomais scheint vor dem Hintergrund abnehmender NDF-Abbaubarkeiten im Vergleich neuerer zu älteren Silomaissorten (Deaville und Givens, 2001) die Verwendung genetischer Ressourcen, die momentan keine Verwendung in der züchterischen Arbeit finden, unausweichlich zu sein (Brichette Mieg et al., 2001; Barrière et al., 2003a). Eine über die chemische Zusammensetzung hinausgehende Beschreibung des Genpools muß vorgenommen werden, wenn nicht ausschließlich auf Verbesserungen von Ertragssicherheit und Stresstoleranz, die erwiesenermaßen als Züchtungserfolge zu bewerten sind, fokussiert werden soll.

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# Kapitel 6

# Zusammenfassung / Summary

## 6.1. Zusammenfassung

Die Futterqualität von Silomais (*Zea mays* L.) wird neben den Ertragsanteilen der einzelnen Pflanzenfraktionen (Kolben, Restpflanze) durch deren chemische Zusammensetzung determiniert. Diese beiden Faktoren sind im Laufe der Abreife systematischen Veränderungen unterworfen, die zum einen durch den Genotyp, zum anderen durch Witterungsbedingungen direkt oder indirekt beeinflusst werden.

Ziel dieser Studie war es demzufolge, (i) repräsentative Silomaissorten anhand der genotypbedingten Gehaltsveränderungen von Qualitätsparametern (wasserlösliche Kohlenhydrate (WLK), Stärke, neutral detergent fiber (NDF), acid detergent fiber (ADF), Hemicellulose, Cellulose, Lignin) in Kolben, Rest- und Gesamtpflanze im Vegetationsverlauf zu charakterisieren und (ii) zu überprüfen, ob witterungsbedingte Effekte mit Hilfe geeigneter Modelle quantifiziert werden können. Ferner wurde (iii) untersucht, ob die Schätzgenauigkeit der Nah-Infrarot-Reflexions-Spektroskopie (NIRS) hinsichtlich der Gasbildung verbessert werden kann.
Datengrundlage der Untersuchung ist ein 3-jähriges Feldexperiment (2001-03), welches im Rahmen des Projektes der 'Regionalen Erntezeitprognose von Silomais' angelegt wurde. Acht Silomaissorten, die das Sortenspektrum hinsichtlich Abreifegruppe (früh, mittelfrüh, mittelspät) und –typ (stay green, dry down, normalabreifend) bzw. Inhaltsstoffzusammensetzung abdecken, wurden an je 6 Terminen (1 vor, 5 nach der Blüte) innerhalb der Vegetationsperiode beprobt. Separate Qualitätsbestimmungen in Kolben und Restpflanze erfolgten auf Basis eigens aufgestellter NIRS-Schätzgleichungen. Die Gesamtpflanzengehalte resultierten aus den Anteilen von Kolben und Restpflanze und ihren entsprechenden Gehalten.

Es zeigt sich, dass die Schätzung der Gasbildung mittels NIRS weiterhin unzulänglich ist. Die Hypothese, dass eine wesentliche Verbesserung der Schätzgenauigkeit durch die Fraktionierung der Gesamtpflanze in Kolben und Restpflanze bzw. durch die Bestimmung der Gasvolumina in Inkubationsintervallen, die mit der Fermentation einzelner Inhaltsstoffe in Verbindung stehen, erzielt werden kann, muss verworfen werden. Die Kalibration zeigt zwar eine zufrieden stellende Schätzgenauigkeit ausgewählter Inkubationsintervalle ($R^2$= 0.87-

0.96; Standardfehler (Kalibration) 0.87-3.17 ml 200 $mg^{-1}$ TM), diese lassen sich jedoch in der Validation nicht bestätigen ($R^2$= 0.35-0.69; Standardfehler (Validation) 1.55-6.06 ml 200 $mg^{-1}$ TM).

Die Varianzanalyse zur Charakterisierung genotypbedingter Unterschiede weist signifikante Sortenunterschiede innerhalb der Reifegruppen sowohl bei Betrachtung der Zellwandbestandteile als auch bei den Gehalten an Nicht-Struktur-Kohlenhydraten auf. In der Gesamtpflanze sind diese vor allem in frühen Entwicklungsstadien ausgeprägt. Zur Siloreife unterscheiden sich die Reifegruppen zumeist signifikant voneinander, wohingegen innerhalb der Reifegruppen nur Unterschiede im WLK-Gehalt der mittelfrühen Silomaissorten abgesichert werden können. Genotypbedingte Differenzen in den Inhaltsstoffgehalten sind daher überwiegend auf die unterschiedlich schnelle Abreife der Sorten zurückzuführen.
Der signifikante Jahreseinfluss auf die Gehalte der Qualitätsparameter kann mit Hilfe der witterungsbasierten Modelle FOPROQ und FONSCH, die ursprünglich für Futtergräser entwickelt wurden, auch für Silomais auf Basis von Tagesdurchschnittstemperatur, Einstrahlung und pflanzenverfügbarem Bodenwasser sortenspezifisch quantifiziert werden. Es waren keine Modifikationen der Modellalgorithmen erforderlich, was ihre generelle Eignung bestätigt. Hohe Bestimmtheitsmaße bis 0.96 (Stärke) in Verbindung mit geringen Fehlern unterstreichen die guten Modellanpassungen.
Anschließende Simulationsstudien zweier ausgewählter Sorten dokumentieren für den Standort Kiel und damit für Grenzlagen der Silomaisproduktion in Norddeutschland eine starke witterungsbedingte Variation, wobei insbesondere die Gehalte an wasserlöslichen Kohlenhydraten eine hohe Umweltabhängigkeit ausweisen. Im Gegensatz dazu wird der Gehalt an Gerüstsubstanzen (NDF, ADF, Cellulose, Hemicellulose) in deutlich geringerem Umfang durch die Umweltbedingungen determiniert. Generell sind die umweltbedingten Variationen stärker ausgeprägt als die genotypischen Differenzen. Witterungsbedingte Unterschiede im NDF, Stärke und WLK-Gehalt in der Gesamtpflanze ausgewählter Silomaissorten belaufen sich auf 1.31-1.66%, 6.56-7.56% bzw. 7.8-10.6%, wohingegen Sortenunterschiede innerhalb der Versuchsjahre 1.65% NDF, 3.97% Stärke und 3.36% WLK nicht überschreiten.

Die hier vorgestellten Untersuchungen ermöglichen eine detaillierte Charakterisierung von Silomaisgenotypen anhand futterwertbestimmender Parameter, v. a. der Kohlenhydratfraktionen. Ferner wird die Bestimmung des Erntezeitpunktes durch Aussagen bezüglich der Qualitätsentwicklung unterstützt. Die Implementierung der Modelle als Entscheidungshilfe für die landwirtschaftliche Beratung erfordert jedoch zuvor noch eine Erweiterung der Datenbasis, d.h. eine größere Zahl unterschiedlicher Umwelten und eine Validation der Modellparameter.

Eine abschließende Bewertung der hier untersuchten Sorten bezüglich ihrer Eignung für die Wiederkäuerernährung kann nicht vorgenommen werden, da Untersuchungen zu Abbaubarkeiten einzelner Inhaltsstoffe nicht vorgenommen wurden.

## 6.2. Summary

Forage quality of silage maize (*Zea mays* L.) is determined by the ear-to-stover as well as by their chemical composition. With advancing maturation of the maize crop, these factors undergo systematic changes, which are mainly driven by genotype and environmental conditions.

The objectives of this study therefore were to characterize (i) the impact of variety and stage of maturity on the contents of quality parameters (water soluble carbohydrates (WSC), starch, neutral detergent fiber (NDF), acid detergent fiber (ADF), hemicellulose, cellulose, and lignin of ear, stover and whole-plant and to investigate, (ii) whether the influence of environmental factors can be quantified by appropriate weather-based models. A further aim was (iii) to explore the potential of near infrared reflectance spectroscopy (NIRS) to predict the gas production of forage maize in order to get an insight of the kinetics of carbohydrate degradation in the rumen.

The study was based on data collected in a 3-year (2001-03) field experiment conducted within the framework of the project 'regional harvest time prediction of silage maize'. Eight maize varieties, covering a wide maturity range (early, mid-early, mid-late) and different maturation types (dry down, normal, stay-green) were investigated. Growth and quality changes of the maize crop were recorded at 6 times (1 pre-, 5 post-silking) throughout the vegetation period. The contents of quality parameters were estimated by NIRS for ear and stover separately with ad hoc calibration and validation. Whole plant contents were derived from corresponding values of stover and ear and their weight proportions.

Efforts to improve NIRS prediction of gas production turned out to be ineffective. The development of calibration equations separately for ear and stover and the consideration of different incubation intervals, which are related to fermentation of specific components, showed promising results for NIRS calibration ($R^2$= 0.87-0.96; standard error (calibration) 0.87-3.17 ml 200 $mg^{-1}$ DM), while validation statistics were poor ($R^2$= 0.35-0.69; standard error (validation) 1.55-6.06 ml 200 $mg^{-1}$ DM). The results indicate that NIRS prediction of gas production needs further improvement.

The results of the analysis of variance revealed genotypic variability for quality parameters. Significant differences between hybrids within a given maturity group were observed for cell wall constituents and non-structural carbohydrates mainly in early stages of development. At silage maturity, however, significant differences within maturity groups were detected for the WSC content of the mid-early group only, while maturity groups differed significantly for most quality traits. Genotypically determined differences therefore are mainly due to differences in the progress of maturation.

The FOPROQ and FONSCH models turned out to be applicable to forage maize without any modifications of its algorithms and allowed to quantify the impact of weather factors on the contents of quality parameters for maize varieties separately. The application of the models allowed to replace the few meaningful factor 'year' by the specific factors temperature, solar radiation, and plant available soil water (precipitation), and to estimate their contribution to quality change. Model calibration showed good agreement between observed and calculated contents, with $R^2$ values up to 0.96 (starch) and low prediction errors. For marginal maize production areas, the field experiment and the results of the simulation study revealed a substantially larger contribution of environmental conditions than genotype effects on the forage quality at silage maturity, especially WSC contents revealed high sensitivity on environmental conditions. Environmentally-caused differences in NDF, starch and WSC contents of the whole-crop of selected cultivars amounted 131-166 g $kg^{-1}$ DM, 656-756 g $kg^{-1}$ DM and 78-106 g $kg^{-1}$ DM, respectively, whereas genotypic differences were below 165 g NDF $kg^{-1}$ DM, 39.7 g starch $kg^{-1}$ DM and 33.6 g WSC $kg^{-1}$ DM.

The results provide the possibility to characterize silage maize genotypes based on quality-related parameters, particularly carbohydrates, and to supplement the determination of harvest time with further details concerning forage quality.

Before introducing the model as a prognosis tool into practical agriculture, the data base underlying model calibration should be extended, in order to improve the reliability of model output with respect to other regions differing in weather conditions. Furthermore, the model requires validation with an independent data set.

Nevertheless, a final assessment of the varieties investigated with respect to their forage quality is not possible because we did not yet analyze the degradability of organic matter components.

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