Evaluation of common and durum wheat rheological quality through Mixolab® analysis after field damage by cereal bugs

Published version:

DOI:10.1016/j.fcr.2015.04.014

FIELD CROPS RESEARCH

Title: Evaluation of common and durum wheat rheological quality through Mixolab® analysis after field damage by cereal bugs.

Authors:

Massimo Blandino[a*], Federico Marinaccio[a], Barbara Letizia Ingegno[a], Marco Giuseppe Pansa[a], Patrizia Vaccino[b], Luciana Tavella[a], Amedeo Reyneri[a]

Affiliation:

[a] University of Torino, Dipartimento di Scienze Agrarie, Forestali e Alimentari, Largo P. Braccini 2, I-10095 Grugliasco (TO), Italy.

[b] Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria, Unità di ricerca per la Selezione dei Cereali e la Valorizzazione delle varietà vegetali (CRA-SCV), via Forlani 3, 26866, S. Angelo Lodigiano (LO), Italy.

*Corresponding author: Massimo Blandino

Phone +39 011 6708895, massimo.blandino@unito.it

Abstract

The pre-harvest damage of wheat by sunn pests decreases flour quality. Mixolab® is a recent instrument that can be used to accurately describe the technological behaviour of flour dough, since it is able to evaluate both protein and starch components at the same time. Two-year field experiments were carried out to study the effect of *Eurygaster maura* (Heteroptera: Scutelleridae) feeding on the quality traits of common and durum wheat, recorded using different protocols, in comparison to the traditional sodium dodecyl sulphate sedimentation test (SSV). In 2012-13 growing season, a damage rate between 16% and 21% of damaged kernels led to a greater reduction in dough stability for common (-65%) than for durum wheat (-32%), while the protein strength was affected more by insect activity in durum wheat (-56%). In 2013-14 growing season, *E. maura* feeding (on average 32% of damaged kernels) reduced SSV by 5% and 66%, dough stability by 12% and 30%, and protein strength by 12% and 16%, for common and durum wheat, respectively. The increasing percentages of damaged kernels in both crops led to a clear decrease in dough stability and protein strength; a significant change in the rheological parameters was noticeable at a 2.5% level of damaged kernels. SSV resulted to be significantly correlated to Mixolab® dough stability, the protein strength, the protein network wakening rate (α) and Change in Mixolab Consistency (CMC, “Wheatbug” protocol) for both common and durum wheat. Therefore, Mixolab® can be considered a suitable tool for a quick detection of damage caused by sunn pests in common and durum wheat flour.

Key words

Common wheat; durum wheat; proteins; gluten; *Eurygaster maura*

Abbreviations: Change in Mixolab Consistency, CMC; Dough Development Time, DDT; Grain Protein Content, GPC; GS, growth stage; Sodium dodecyl sulphate sedimentation volume, SSV; water absorption, WA.

1. Introduction

Grain protein content (GPC) and gluten quality are key traits that influence the end-use of wheat market classes throughout the world (Foca et al., 2007). High levels of proteins as well as gluten quantity and strength are the predominant factors associated with superior bread- and pasta-making quality. Therefore, these traits are desirable for the marketability of both common and durum wheat in several supply chains (Brown and Petrie, 2006; Kovacs et al., 1997). Although the environmental conditions and the genotype, i.e. the choice of variety, are fundamental for the technological behaviour of the derived dough, the agricultural practices, and in particular nitrogen nutrition, also influence the flour quality to a greater extent.

Considerable modifications of wheat quality can also be produced by several species of insects, in particular cereal bugs, which are also known as sunn pests, belonging to Scutelleridae (shield-backed bugs) and Pentatomidae (stink bugs) (Critchley, 1998). *Eurygaster maura* (L.) (Heteroptera: Scutelleridae) is the most noxious species in western Europe, in terms of reduction in grain quality (Pansa et al., 2015; Vaccino et al., 2006). Sunn pests feed on wheat during the different stages of developing grains; in case of early attacks, the damage mainly concerns losses in kernel weight and consequently in grain yield; late attacks during the grain filling period, which are more frequent, lead to a reduction in technological quality. In this case, the insects suck the milky nutrients from the immature grain by piercing it and injecting proteolytic enzymes, via their saliva, which persist in the flour after milling and cause the breakdown of the gluten structure in the dough (Olanca et al., 2009). Thus, the rheological properties of the dough obtained from bug-damaged wheat are characterized by lower farinographic development time and stability, lower alveographic strength, tenacity and extensibility, and a lower gluten index (Karababa and Ozan, 1998).

In the case of common wheat, the result is the production of bread with poor volume and texture (Aja et al., 2004; Pérez et al., 2005), while in the case of durum wheat, bug damage can negatively affect the cooking potential, especially in varieties with poor gluten quality (Petrova, 2002). Ozderen et al. (2008) have demonstrated that the semolina properties and spaghetti quality of durum flour obtained from bug-damaged wheat decreased significantly as the damage levels increased. Although there are several reports on the effects of bug damage on common wheat in the literature, limited information is available related to its effects on durum wheat, and in particular a direct field comparison of the bug feeding effect on both crops is missing.

The relevant reduction in technological quality of both common and durum wheat underlines the necessity of bug damage control before batch processing. The practical tolerance for bug-damaged kernels in industry, regardless of the wheat type (common or durum) or variety, is 2-3% (Canhilal et al., 2006). The damage caused by sunn pests can be detected through a visual inspection of the kernels which are characterized by a discoloured halo around the stylet penetration point (Critchley, 1998). Unfortunately, visual damage detection is often not completely reliable, since it is strongly related to the operator’s experience and sensitivity. Moreover, similar symptoms can be sometimes due to the activity of pathogens or the abnormal starch deposition. As a consequence, visual inspection should be associated with complex biochemical analyses which assess protein degradation due to insect attack, such as reverse-phase or size-exclusion high performance liquid chromatography and free zone capillary or gel electrophoresis (Sivri et al., 1999; Aja et al., 2004; Rosell et al., 2002a; Vaccino et al., 2006).

At the technological level, one of the most specific methods to detect bug damage on grains and flour is the sodium dodecyl sulphate sedimentation volume test (SSV) proposed by Every (1992).

According to the European ISO605 and the United States Department of Agriculture (USDA) standards, the food supply chain requires practical and reliable screening procedures in order to ensure the technological quality and marketability of wheat kernels and flour. Mixolab® (Chòpin Technologies, Villeneuve la Garenne, France) is a recent instrument that is used to determine the rheological quality of flour and to more accurately describe its behaviour during bread making. This device provides, in one single test, a complex analysis of the rheological properties of wheat flour dough, considering dough behaviour during mixing, protein coagulation, heating-up behaviour at enzyme activity intensification, and starch gelatinization and retrogradation during the final cooling. The instrument has also proved useful to analyse the quality of durum wheat (Moscaritolo et al., 2008). Kahraman and Köksel (2013a) have recently suggested a new and specific Mixolab® analytical protocol to estimate bug damage in flour.

The aims of this study were to make the direct field comparison of the bug feeding effect on both common and durum wheat and to evaluate the potential of Mixolab® to detect bug damage on the two crops considering different analysis protocols.

2. Material and Methods

2.1. Agronomic information

Field trials were carried out in Carignano, Piedmont, NW Italy (44°53'8.69"N, 7°41'16.75"E, 232 m a.s.l.), in a medium-texture fertile soil, during the 2012-13 and 2013-14 growing seasons. Common and durum wheat were cultivated side by side in the same field, according to the normal crop management programme applied to wheat in the growing area.

The common wheat cultivars were Generale (Consorzio nazionale sementi, Conselice, RA, Italy) in 2012-13 and Arrocco (Limagrain Italia S.p.A., Busseto, PR, Italy) in 2013-14, which are classified, according to the Italian bread-making quality grade (Foca et al., 2007), as superior bread making-quality wheat. The durum wheat cultivar were Colombo (Apsovsementi S.p.A., Voghera, PV, Italy) in 2012-13 and Saragolla (Produttori Sementi Bologna S.p.A., Argelato, BO, Italy) in 2013-14, which are classified as high quality wheat. Planting was conducted in 12 cm wide rows at a seeding rate of 450 seeds m^{-2} on the last decade of October. The previous crop was maize for grain every growing season. The weed control was conducted with isoproturon and diflufenican at wheat tillering (growth stage, GS 23; Zadoks et al., 1974), while fungicide treatments were performed to avoid the development of foliar and head fungal diseases at stem elongation (GS 35, a.i. azoxystrobin and cyproconazole applied at 0.2 kg ha^{-1} and 0.08 kg ha^{-1}, respectively) and at heading (GS 58, a.i. prothioconazole applied at 0.250 kg ha^{-1}). A total of 170 kg N ha^{-1} was applied as a granular ammonium nitrate fertilizer, split 50 kg N ha^{-1} at wheat tillering (GS 23), 80 kg N ha^{-1} at stem elongation (GS 32) and 40 kg N ha^{-1} at booting (GS 46).

2.2. Collection and rearing of cereal bugs

During spring 2013 and 2014, large quantities of *E. maura* were collected in several wheat fields in Piedmont, and transferred to laboratories. There, they were reared on kernels and small wheat plants inside 3 L plastic boxes. The lid of these boxes (265 mm × 175 mm) was cut in the middle and closed with a net. Mass rearing was conducted in climatic chambers maintained at 25 ± 1°C, 70 ± 5% RH and a 16L:8D photoperiod.

2.3. Exposure of wheat to bug feeding

Two parallel experiments were performed in order to evaluate the effects of bug feeding on common and durum wheat quality. These experiments involved comparing the rheological parameters of the flour derived from: i) grain samples obtained in the presence or absence of *E. maura* in field conditions (first experiment); ii) grain samples with different percentages of bug-damaged kernels (second experiment).

The first experiment was conducted during two growing seasons using large net cages (4×4×3.5m) which were placed at early milk stage (GS 73) for both crops, after a careful check for the absence of bugs. Two treatments, that are the presence of *E. maura* and control without insects, and three replications for each treatment and crop were performed according to a completely randomized experimental design. In 2013, 48 individuals (i.e., 3 individuals m^{-2}) were introduced into each cage and left until the end of wheat ripening (July 2). In 2014, 45 individuals were introduced into each cage and left until the end of wheat ripening (July 1). At this stage, in both years, all the bugs were removed and recorded through an accurate inspection of the cage contents.

On July 22, 2013 and July 11, 2014, the grain was obtained by harvesting with a Walter Wintersteiger cereal plot combine-harvester, and 2 kg grain samples were taken from each cage for the qualitative analyses. Damaged kernels, i.e. the percentage of seeds showing, on visual inspection, the typical discoloured area around the point of bug stylet penetration, were determined on three 100-kernel randomly selected samples for each treatment and replication.

In the second experiment, carried out in 2012-13 growing season, white sleeve polythene fine mesh net cages were positioned in the field for both crops at early maturity stage. In particular, 25 cages (0.5 m long, 0.2 m diameter) were suspended from a pole in the ground, and the cage ends were closed over the vegetation to prevent entry and escape of the cereal pest. On June 5, at early milk stage (GS 73), a group of 20 spikes was included into each cage, and 1 adult, 5 3rd-instar juveniles and 35 eggs were introduced, left for 27 days, and removed at the wheat ripening, in order to obtain high amounts of bug-damaged kernels. The spikes from each cage were hand harvested, placed in paper bags, and transferred to laboratories where they were threshed with a Minibat Godé laboratory-sheller (Godé, Le Catelet, France). The kernels were visually inspected to detect and keep only those showing an evident dark puncture mark at the bug stylet penetration point, surrounded by a discoloured area.

Five mixtures of sound kernels were obtained for each crop with an increasing replacement rate (1%, 2.5%, 5%, 10%, 20%) of bug-damaged kernel, and, after an accurate mixing, the kernels were employed for milling. Two replications of 600 g of kernels were obtained for each replacement level. The undamaged kernels were obtained from pre-defined field plots in which a pyrethroid insecticide (a.i.

deltamethrin applied at 0.012 kg ha^{-1}) had been applied at the beginning of the milk stage (GS 71). These sound kernels were analyzed as a control.

2.4. Quality analyses

Grain moisture content was determined by means of a Dickey-John GAC2100 grain analysis meter (Dickey-John Corp., Auburn, IL, USA) using the supplied programme. Moisture calibration was checked using oven drying techniques.

Grain samples (300 g) from each plot were milled using a Retsch ZM 200 (Retsch GmbH, Haan, Germany), fitted with a 1 mm aperture sieve, and the resulting wholemeal was analysed by near-infrared reflectance spectroscopy, using a NIRSystems 6500 monochromator (Foss-NIRSystems, Silver Spring, MD, USA). The GPC (N x 5.7, dry matter basis) was determined according to AACC 39-10 (AACC, 2000).

Two kg and 0.6 kg grain samples were milled for each replication, for the first and second experiments, respectively, with an experimental mill (Labormill 4RB, Bona, Monza, Italy) after tempering according to the grain hardness. The applied milling operation conditions, in particular the type of rolls and sieves, were specific for each crop, common and durum wheat crop, according to the experimental mill protocol.

The rheological properties were studied on each flour sample using a Mixolab® analyser (Chòpin Technologies, Villeneuve la Garenne, France), according to the ICC Standard Method 173 (ICC, 2010). This instrument allows specific information to be obtained on the behaviour of dough constituents (starch, protein, water) by continuously measuring the torque (Nm) produced as the dough passes between two mixing blades and is subject to the dual stress of mixing and temperature changes. The test is based on the preparation of a constant dough sample to obtain a target consistency (1.1 Nm +/-0.05) during the first test phase. Samples were mixed choosing the “Chopin+” protocol. The resulting Mixolab® curve is separated into five different phases. A detailed description of the physical changes that occur during a Mixolab® measurement (Chopin+ protocol) was reported in Rosell et al. (2007). The key parameters derived from the Mixolab® curve are: water absorption capacity (WA, %), which represents the amount of water that flour can absorb to obtain a pre-set dough consistency to produce a torque (peak C1) of 1.1 Nm +/-0.05 at 30°C; Dough Development Time (DDT, min), that is the time required to obtain the maximum torque (C1) at 30°C; dough stability (min) or elapsed time, at which the produced torque is kept at 1.1 Nm (± 11%); amplitude (Nm), which represents the width of curve to C1 and refers to dough elasticity; C2 (Nm), which measures protein strength after a decrease in dough consistency due to mechanical shear stress and temperature increase (from 30°C to 60°C) and provides an indication of protein quality; C3 (Nm), which measures starch gelatinization (starch granules absorb water and the further increase in temperature, from 60°C to 90°C, results in an increase in viscosity); C4 (Nm), which measures hot gel stability connected to the amylase activity (at a constant temperature of 90°C); C5 (Nm), which measures starch retrogradation in the cooling phase (from 90°C to 50°C); α, the slope of the curve between the end of the period of 30°C and C2, and refers to the protein network weakening rate (nm/min); β, the gelatinization rate (Nm/min) or the slope of the curve between C2 and C3; γ, the cooking stability rate (Nm/min) or the slope of the curve between C3 and C4.

In addition, flour samples were mixed applying the Mixolab “Wheatbug” protocol, as proposed by Kahraman and Köksel (2013b). Briefly, the protocol is characterized by a mixing period of 3 min at 110 rpm, incubation for 20 min without mixing and a mixing period of 5 min at 110 rpm at a constant temperature of 35°C. The decrease in consistency between the 2.9th min and the 28th min (CMC: Change in Mixolab Consistency) was calculated and expressed as a percentage.

A sodium dodecyl sulphate sedimentation volume test (SSV) was performed on the same flour samples analysed with Mixolab®, according to Every (1992).

2.5. Statistical analyses

An analysis of variance (ANOVA) was used to compare the qualitative parameters recorded in both experiments.

The residual normal distribution was verified using the Kolmogorov-Smirnov test, while variance homogeneity was verified using the Levene test. In the second experiment, multiple comparison tests were performed according to the Ryan-Einot-Gabriel-Welsch F test on treatment means.

Simple correlation coefficients were obtained for all the Mixolab® parameters, relative to each other and to SSV, while keeping the data sets together which referred to the two experiments carried out in 2012-13 growing season, but considering common and durum wheat separately. The relationship between the best Mixolab parameters correlated to bug feeding damage and SSV was studied for each crop through linear regression analysis.

The SPSS for Windows statistical package, Version 21.0 (SPSS Inc., Chicago, IL, USA), was used for the statistical analyses.

3. Results

In the first experiment, a direct comparison of qualitative damage to common and durum wheat exposed to *E. maura* was made in field conditions. No significant differences (ANOVA, $P>0.05$) in the number of bugs captured in the cages were found between the two crops in 2012-2013 (76.67±27.67 for common wheat and 62.00±19.86 for durum wheat) and in 2013-2014 (145.67±15.38 for common wheat and 115.33±18.89 for durum wheat).

The results of artificial infestation with *E. maura*, which was conducted for direct comparison with an uninfested control in field conditions for 2012-13 and 2013-14 growing seasons on damaged kernel rate, GPC and the Mixolab parameters are reported in Table 1.

In 2012-13 growing season, ANOVA showed a significant effect of the insect infestation on the damaged kernel percentage, SSV, dough stability, C2, C3, C4 and C5 points, α and β slopes, and CMC for both crops. The *E. maura* infestation also significantly affected the WA and γ slope, but only for the durum wheat. The GPC was not significantly different between the compared treatments for either crops.

On visual inspection, the average percentage of damaged kernels, after the artificial infestation of *E. maura*, was 21.6% and 16.0% for common and durum wheat, respectively. Compared to the control, which was characterized by less than 1% of kernels with visual symptoms, insect feeding reduced SSV by 40% and 68%, for common and durum wheat, respectively. As far as the Mixolab® parameters of Chopin+ protocol are concerned, the bug damage led to a greater reduction in dough stability for common (-65%) than durum wheat (-32%). On the other hand, the parameters that describe the protein strength (the C2 point and α slope) were affected more by insect activity in the durum wheat. The observed reductions in the

C2 value were 40% and 56%, while those of the α value were 39% and 44%, for common and durum wheat, respectively. C3, C4 and C5, which describe starch behaviour to temperature modification, also resulted in a greater decrease, as a result of the insect presence, in durum than in common wheat. When considering the Wheatbug Mixolab® protocol, the insect feeding activity increased the CMC by 34% and 24% for common and durum wheat, respectively.

In 2013-14 growing season, ANOVA confirmed a significant effect of the insect infestation on the damaged kernel percentage, SSV, dough stability, C2 and CMC for both crops. Although the average percentage of damaged kernels, after the artificial infestation of *E. maura*, was higher compared to that observed in the previous growing season, the rheological impact measured through SSV or Mixolab® test was generally lower. Compared to the control, insect feeding reduced SSV by 5% and 66%, dough stability by 12% and 30%, and C2 value by 12% and 16%, for common and durum wheat, respectively. Moreover, the insect feeding activity increased the CMC by 12% and 11% for common and durum wheat, respectively.

In the second experiment, the increasing percentages of damaged kernels (i.e., with visual damage of bug feeding) led to a significant decrease in SSV, dough stability, the C2 and C3 values, and CMC for both common and durum wheat (Table 2). For both crops, the effect of a reduction in protein quality, measured through the SSV test, began at 1% of replacement with kernels damaged by insect feeding. The SSV values at 1% of damaged kernels were 7% and 11% lower than the control (i.e., sound samples with a 0% replacement level), for common and durum wheat, respectively. The durum showed a greater reduction in SSV at each replacement level compared to common wheat, thus underlining a probable higher susceptibility of this crop to the *E. maura* feeding activity on grains.

As far as the Mixolab® Chopin+ protocol is concerned, dough stability and the C2 value were confirmed to be the most closely related parameters to bug damage for both crops, although they showed a lower capacity to stress the insect damage than SSV. A significant reduction in dough stability and protein strength (C2) was observed for common wheat at the 2.5% and 5% replacement levels, respectively. On the other hand, the C2 value was significantly lower for durum wheat than the control at 2.5%, while only at 5% of substitution did the dough stability significantly decrease. At 5% of kernel substitution, the dough stability was reduced by 33% and 25% for common and durum wheat, respectively. The reduction in protein strength (C2) was 12% and 28% for common and durum wheat, respectively at the same rate. Starch gelatinisation (C3) was affected by the percentage of bug-damaged kernels at the 5% level for common wheat, while it was only influenced significantly at the 20% level for durum wheat.

Conversely, C4, C5 and β varied significantly with the increase in damaged kernels for common wheat, but the differences in these parameters were only more pronounced for the highest replacement levels. Only for durum wheat, but at 20% of substitution, did the protein network wakening rate (α) show a significant difference, compared to the control. The WA, DDT, amplitude and cooking stability rate (γ) were unaffected by the percentage of damaged kernels.

The CMC parameter obtained using the Wheatbug protocol was confirmed to be well able to describe bug damage and showed similar results to those observed with the Chopin+ protocol for dough stability and the C2 value: the analysis showed a significantly higher CMC at 2.5% of substitution compared to the control for both crops.

Table 3 reports the correlation coefficients and their significances for the Mixolab® parameters and SSV for flour samples derived from both the first and the second experiment carried out on cvs Generale and Colombo in 2012-13 growing season. SSV resulted to be significantly correlated to dough stability, the protein strength (C2), the protein network wakening rate (α) and CMC for both common and durum wheat. The correlation was always highly significant ($P<0.01$), with the exception of α for common wheat ($P<0.05$). Moreover, a significant correlation was observed between SSV and C3, C4, C5, β and γ, but only for common wheat. As expected, the different Mixolab® parameters were often significantly correlated to each other.

The relationship between SSV and dough stability, the C2 value, the α value and CMC is shown in Figure 1 for both considered crops. The regression analysis for the reported equations was always significant. The highest R^2 values were observed for the relationship between SSV and protein strength (C2) for both common ($R^2 = 0.87$, $P<0.01$) and durum ($R^2 = 0.93$, $P<0.001$) wheat.

4. Discussion

The present study underlines that Mixolab® can properly estimate and describe damage caused by sunn pests in both common and durum wheat. The occurrence of enzymatic degradation of the gluten proteins, caused by *E. maura*, clearly affected the parameters that are closely related to gluten content and composition: dough stability, protein strength (C2), protein network wakening rate (α). These changes in dough rheological properties are related to a loss in gluten functionality, resulting from complex changes in the gluten structure (Pérez et al., 2005; Torbica et al., 2014). The previously reported Mixolab® parameters are closely correlated to SSV and were clearly able to describe the occurrence of bug damage in each wheat species. The Mixolab® parameter most closely related to bug damage, expressed as SSV, for both considered crops was protein strength.

Moreover, a clear effect of insect infestation was also observed on starch gelatinization (C3), hot gel stability (C4), starch retrogradation in the cooling phase (C5) and starch gelatinisation rate (β) for both crops, particularly in the first experiment for the 2012-13 growing season. Conversely, in the second experiment, considering the different replacement levels, the impact of the increasing percentage of bug-damaged kernels on the previously reported starch parameters was more evident on common wheat than on durum wheat. The effects of the alteration of starch granules have already been reported for *Nysius* spp. (Heteroptera: Lygaeidae), a bug species noxious to wheat, in Australia and New Zealand (Every et al., 1990; Lorenz and Meredith, 1988), although no differences in diastatic activity were assessed. However, amylase activity has not been reported to be involved in wheat damage caused by *Aelia* spp. or *Eurygaster* spp., and starch granules examined by means of scanning electron microscopy were also intact in the surrounding areas of the insect puncture (Rosell et al., 2002b). Thus, it can be supposed that the observed different dough behaviour, concerning the starch quality Mixolab® parameters, is a consequence of gluten alteration due to *E. maura* in the endosperm protein-starch matrix rather than a direct effect on starch granules.

Although the quality of gluten, as well as the quantity, has a clear impact on dough water absorption and development time (Cauvain and Young, 2000), the occurrence of *E. maura* damage, obtained through an artificial infestation or by increasing substitution of damaged kernels, did not here result in an impact on WA or DDT, with the exception of the WA of durum wheat in the first experiment in the 2012-13 growing season. This result is in contrast with the results of Hariri et al. (2000), who reported a proportional increase in WA and decrease in DDT for a rise in the damaged kernel percentage.

As far as the specific Mixolab® Wheatbug protocol is concerned, the CMC parameter seems to provide clear evidence of bug damage, especially for common wheat, which results in a higher correlation to dough stability and protein strength (C2) than SSV. Conversely, for durum wheat, the relationship between the Mixolab® rheological parameters and SSV is closer than the relationship between these parameters and CMC.

A close positive relationship between bug density and the percentage of kernel damage in common and durum wheat was reported in previous studies on *E. integriceps* Puton (Heteroptera: Scutelleridae), along with a negative relationship between increasing bug-damaged kernels and flour quality parameters (Canhilal et al., 2006; Karababa and Ozan, 1998). The present research has shown that the detrimental effects of bug damage on the rheological quality of durum wheat are quite similar to those that occur on common wheat. These data, obtained by means of the Mixolab® analysis, confirm the results of Olanca and collaborators (2009) who considered the impact of sunn pests on protein composition and on the structure of durum wheat. However, the effect of *E. maura* on the SSV parameter was generally higher in durum wheat than in common wheat, considering both growing seasons and different cultivars. Comparing different common wheat varieties, Kinaci and Kinaci (2004) demonstrated a variation in quality damage due to sunn pests among wheat varieties. The authors reported that, in terms of sedimentation values, hard grains suffered more than soft ones after bug infestations.

Mixolab® pointed out significant differences on the parameters of dough stability, protein strength and CMC in both growing seasons and for both crops, even considering different cultivars. However, in the 2013-14 growing season the rheological impact, measured through SSV or Mixolab® dough stability, protein strength and CMC test, was lower even if the average percentage of damaged kernels was higher compared to that observed in the previous growing season.

This effect should not be related only to the use of different cultivars, which have similar level of quality. A possible explanation could be related to the meteorological conditions that occurred during wheat ripening: the 2013-14 growing season was characterized by frequent rainfall and lower temperatures from flowering to harvesting compared to the 2012-13 season. These conditions may have contributed to change the quality of kernel proteins or led to a higher fungal kernel infection, with an overestimation of visual insect damage. Nevertheless, both SSV and Mixolab® evaluations rely upon the content and quality of kernel proteins, which is related to the environmental and agronomic conditions; as a consequence, such methods only indirectly and non-specifically define gluten damage caused by bugs. A specific quantification of damage caused by bugs should be addressed with biochemical methods able to assess protein degradation due to insect attack. An immunological method for a quick and direct detection of bug contamination on flour batches, as preliminarily reported (Vaccino et al., 2008), could be a specific and effective tool to detect bug damage.

As far as the tolerance of bug-damaged kernel is concerned, this study has also confirmed for durum wheat results obtained in other studies referring to the effect of bug-damaged kernel percentage to dough quality parameters of common wheat. Karababa and Ozan (1998) and Hariri et al. (2000), in experiments carried out in Turkey and Syria, respectively, reported the physicochemical properties (farinograph stability time, mixograph development time) of common wheat samples with more than 5% bug-damaged kernels changed remarkably and showed significantly lower bread-making quality. In the present experiments, the percentage of bug-damaged kernels clearly affected the quality of both common and durum wheat, although the main rheological parameters (dough stability for common wheat, C2 for durum wheat) and CMC obtained with the Mixolab® Wheatbug protocol were already noticeable at the 2.5% addition of damaged kernel level. These results confirm the study of El-Haramain et al. (1984), who reported that a 2-3% grain damage clearly reduces the acceptability of flour for baking purposes. It can therefore be seen that the SSV analysis has been able to stress a significant difference, compared to the control even at a 1% replacement level with damaged kernels, thus confirming the remarkable ability of this test to detect the bug damage of flour.

Ozderen et al. (2008) reported, for durum wheat, that the pasta processing industry could tolerate a relatively higher level of bug damage than the bread processing industry. Furthermore, the authors compared quite high bug damage levels (around 20% and 40%) with a sound control (0%), with reference to spaghetti cooking quality.

The collected data suggest that for both wheat species (common and durum) the presence of 2.5-5% of damaged kernels is sufficient to seriously affect their quality.

Petrova (2002) reported that, depending on the variety, bug damage negatively affected the cooking quality of durum wheat: no significant changes were observed for high gluten quality cultivars, even at a 5% damage level, while a 2% damage level affected pasta quality in weak gluten cultivars.

In conclusion, Mixolab® can be considered a suitable tool for a quick detection of damage caused by sunn pests in common and durum wheat, as it shows a clear correlation to the percentage of damaged kernels. The reduction in rheological quality evaluated through this device was similar for both crops, although the detrimental effects of bug damage on the SSV parameter were greater in durum wheat than in common wheat in both growing seasons.

Acknowledgements

The authors would like to thank Alessandra Gazzola, Matteo Calcagno and Valentina Sovrani for their precious help and cooperation in the field and laboratory work.

The research was conducted with the financial support of the Regione Piemonte, as part of the QUALICHAIN project.

References

AACC International, 2000. Approved methods of the American association of cereal chemists, 10th edn, AACC Eds., St Paul, Minnesota.

Aja, S., Pérez, G., Rosell, C.M., 2004. Wheat damage by *Aelia* spp. and *Eurygaster* spp.: effects on gluten and water-soluble compounds released by gluten hydrolysis. J. Cereal Sci. 39, 187-193.

Brown, B.D., Petrie, S., 2006. Irrigated hard winter wheat response to fall, spring, and late season applied nitrogen. Field Crop. Res. 96, 260-268.

Canhilal, R., Kutuk, H., Kanat, A.D., Islamoglu, M., El-Haramein, F., El-Bouhssini, M., 2006. Economic threshold for the sunn pest, *Eurygaster integriceps* Put. (Hemiptera: Scutelleridae), on wheat in southeastern Turkey. J. Agr. Urban Entomol. 22(3-4), 191-201.

Cauvain, S.P., Young, L.S., 2000. Bakery Food Manufacture and Quality: Water Control and Effects. Blackwell Science, Oxford, pp. 224.

Critchley, B.R., 1998. Literature review of sunn pest *Eurygaster integriceps* Put. (Hemiptera, Scutelleridae). Crop Prot. 17, 271-287.

El-Haramain, F.J., Williams, P., Rashawani, A., 1984. A simple test for the degree of damaged caused in wheat by suni bug (*Eurygaster* spp.) infestation. Rachis. 3, 11-17.

Every, D., 1992. Relationship of bread baking quality to levels of visible wheat-bug damage and insect proteinase activity in wheat. J. Cereal Sci. 16, 183-193.

Every, D., Farell, J.A.K., Stufken, MW, 1990. Wheat-bug damage in New Zealand wheats: the feeding mechanism of *Nysius huttoni* and its effect on the morphological and physiological development of wheat. J. Sci. Food. Agric. 50, 297300.

Foca, G., Ulrici, A., Corbellini, M., Pagani, M.A., Lucisano, M., Franchini, G.C., Tassi, L., 2007. Reproducibility of the Italian ISQ method for quality classification of bread wheats: An evaluation by expert assessors. J. Sci. Food. Agric. 87, 839-846.

Hariri, G., Williams, P., El-Haramein, F.J., 2000. Influence of pentatomid insects on the physical dough properties and two-layered flat bread baking quality of Syrian wheat. J. Cereal Sci. 31, 111–118.

ICC, 2010. Standard Methods of the International Association for Cereal Chemistry 173. International Association for Cereal Science and Technology, Vienna.

Kahraman, K., Köksel, H., 2013a. A new tool to estimate the suni-bug damage in wheat: Mixolab. Proceedings of EUROFOODCHEM XVII, May 7–10, 2013, Istanbul, Turkey, Köksel H. Ed., pp. 581.

Kahraman, K., Köksel, H., 2013b. Estimation of protease activity by use of Mixolab. In: Mixolab: A new approach to rheology, Dubat A., Molina-Rosell C., Gallagher E. (Eds.), AACC International Press, St. Paul, MN, pp. 59-64.

Karababa, E., Ozan, A.N., 1998. Effect of wheat bug (*Eurygaster integriceps*) damage on quality of a wheat variety grown in Turkey. J. Sci. Food. Agric. 77, 399-403.

Kinaci, E., Kinaci, G., 2004. Quality and yield losses due to sun pest (*Hemiptera*: Scutelleridae) in different wheat types in Turkey. Field Crop. Res. 89, 187-195.

Kovacs, M.I.P., Poste, L.M., Butler, G., Woods, S.M., Leisle, D., Noll, J.S., Dahlke, G., 1997. Durum wheat quality: comparison of chemical and rheological screening tests with sensory analysis. J. Cereal Sci. 25, 65-75.

Lorenz, K., Meredith, P., 1988. Insect-damaged wheat effects on starch characteristics. Starch 40, 136-139.

Moscaritolo, S., Amoriello, T., D'Egidio, M.G., 2008. Valutazione delle caratteristiche reologiche di impasti in frumento duro [New approach to evaluate the rheological characteristics of durum wheat doughs]. Tecnica Molitoria 59, 343-352.

Olanca, B., Ozay, D.S., Koksel, H., 2009. Effects of suni-bug (*Eurygaster* spp.) damage on size distribution of durum wheat (*Triticum durum* L.) proteins. Eur. Food Res. Technol. 229, 813-820.

Ozderen, T., Olanca, B., Sanal, T., Ozay, D.S., Koksel, H., 2008. Effects of suni-bug (*Eurygaster* spp.) damage on semolina properties and spaghetti quality characteristics of durum wheats (*Triticum durum* L.). J. Cereal Sci. 48, 464-470.

Pansa, M.G., Blandino, M., Ingegno, B.L., Ferrari, E., Reyneri, A., Tavella, L., 2015. Toxicity and persistence of three pyrethroids for the control of cereal bugs on common wheat. J. Pest Sci. 88, 201-208.

Pérez, G., Bonet, A., Rosell, C., 2005. Relationship between gluten degradation by *Aelia* spp and *Eurygaster* spp and protein structure. J. Sci. Food. Agric. 85, 1125-1130.

Petrova, I., 2002. Effect of bug damage on cooking potential of Bulgarian durum wheat cultivars depending on their gluten strength. . Bulg. J. Agric. Sci. 8, 245-250.

Rosell, C.M., Aja, S., Bean, S., Lookhart, G., 2002a. Effect of *Aelia* spp. and *Eurygaster* spp. damage on wheat proteins. Cereal Chem. 79, 801-805.

Rosell, C.M., Aja, S., Sadowska, J., 2002b. Amylase activities in insect (*Aelia* and *Eurygaster*)-damaged wheat. J. Sci. Food. Agric. 82, 977-982.

Rosell, C.M., Collar, C., Haros, M., 2007. Assessment of hydrocolloid effects on the thermo-mechanical properties of wheat using the Mixolab. Food Hydrocolloids 21, 452-462.

Sivri, D., Sapirstein, H.D., Köksel, H., Bushuk, W., 1999. Effects of wheat bug (*Eurygaster maura*) protease on glutenin proteins. Cereal Chem. 76, 816-820.

Torbica, A.M., Mastilović J.S., Pojić, M.M., Ževrešan Z.S., 2014. Effects of wheat bug (*Eurygaster* spp. and *Aelia* spp.) infestation in preharvest period on wheat technological quality and gluten composition. The Scientific World Journal, Article ID 148025, 6 pages, DOI:10.1155/2014/148025.

Vaccino, P., Corbellini, M., Reffo, G., Zoccatelli, G., Migliardi, M., Tavella, L., 2006. Impact of *Eurygaster maura* (Heteroptera: Scutelleridae) feeding on quality of bread wheat in relation to attack period. J. Econ. Entomol. 99, 757-63.

Vaccino, P., Guidone, L., Corbellini, M., Tavella, L., 2008. Detection of damage due to bug feeding on hazelnut and wheat by biochemical techniques. Bull. Insectology 61, 189-190.

Zadoks, J.C., Chang, T.T., Konzak, C.F.,1974. A decimal code for the growth stages of cereals. Weed Res. 14, 415-421.

Tables

Table. 1

Effect of cereal bug infestation on damaged kernel, grain protein content (GPC), sodium dodecyl sulphate sedimentation volume (SSV) and Mixolab parameters of flour and semolina derived from common and durum wheat, respectively, in 2012-13 and 2013-14 growing seasons.

Year	Crop	*E. maura*	Damaged			Chopin+ protocol[a]										Wheatbug protocol[b]
		infestation	kernels (%)	GPC (%)	SSV (ml)	WA (%)	DDT (min)	Amplitude (Nm)	Stability (min)	C2 (Nm)	C3 (Nm)	C4 (Nm)	C5 (Nm)	α (Nm/min)	β (Nm/min)	γ (Nm/min)	CMC (%)
2013	Common wheat	Control	0.8	14.0	58	56.9	1.8	0.08	10.0	0.54	2.09	1.90	3.11	-0.09	0.69	-0.04	18
		Artificial infestation	21.6	13.9	38	56.8	1.7	0.08	3.5	0.32	1.76	1.66	2.65	-0.05	0.17	-0.04	52
		P (F)	**< 0.001**	0.896	**< 0.001**	0.141	0.212	0.667	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	0.935	**< 0.001**
		sem[c]	2.0	0.4	1.7	0.1	0.2	0.01	0.428	0.019	0.03	0.04	0.09	0.03	0.08	0.03	1.8
	Durum wheat	Control	0.6	13.4	36	58.8	1.3	0.11	3.2	0.28	1.25	0.72	1.17	-0.07	0.32	-0.07	41
		Artificial infestation	16.0	13.8	13	63.0	1.2	0.10	2.2	0.12	0.92	0.55	0.86	-0.04	0.24	-0.05	65
		P (F)	**< 0.001**	0.102	**< 0.001**	**< 0.001**	0.137	0.291	**0.018**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**0.007**	**0.046**	**< 0.001**
		sem[c]	2.2	0.2	1.7	0.8	0.1	0.01	0.7	0.01	0.03	0.04	0.09	0.02	0.04	0.01	1.4
2014	Common wheat	Control	1.67	14.5	87.7	57.8	2.47	0.07	10.33	0.58	2.23	1.70	3.36	-0.06	0.41	-0.29	12
		Artificial infestation	27.9	14.3	82.9	57.8	2.35	0.08	9.07	0.51	2.09	1.62	3.46	-0.04	0.40	-0.14	23
		P (F)	**< 0.001**	0.346	**< 0.001**	0.778	0.590	0.101	**0.019**	**< 0.001**	**< 0.001**	0.126	0.449	0.640	0.925	0.374	**< 0.001**
		sem[c]	4.6	0.3	0.70	0.31	0.31	0.01	0.47	0.02	0.01	0.06	0.16	0.03	0.20	0.21	1.8
	Durum wheat	Control	2.4	13.9	65.3	64.8	1.41	0.11	3.96	0.25	0.96	0.25	0.43	-0.06	0.22	-0.06	44
		Artificial infestation	35.9	13.9	22.3	64.2	1.29	0.10	2.76	0.21	0.95	0.17	0.30	-0.03	0.21	-0.05	54
		P (F)	**< 0.001**	0.877	**< 0.001**	0.207	0.220	0.519	**0.021**	**0.028**	0.704	0.200	0.307	0.102	0.502	0.686	**0.027**
		sem[c]	2.3	0.3	3.13	0.60	0.11	0.01	0.46	0.02	0.03	0.05	0.11	0.01	0.02	0.01	4.4

Reported values are based on 3 replications.

[a] Mixolab parameters Chopin+ protocol: WA = water absorption, the amount of water required in dough development; DDT = Dough Development Time; stability = time of dough stability at constant temperature; amplitude = dough elasticity; C2 = protein strength; C3 = starch gelatinisation; C4 = hot gel stability; C5 = starch retrogradation in the cooling phase; α = protein network weakening rate ; β = starch gelatinisation rate; γ = cooking stability rate. C2, C3, C4 and C5: end points of the corresponding mixing stages.

[b] Mixolab parameter “Wheatbug” protocol: CMC = Change in Mixolab Consistency

[c] sem = standard error of mean.

Table. 2

Effect of the replacement level of common and durum wheat kernels with cereal bug damaged ones on sodium dodecyl sulphate sedimentation volume (SSV) and the Mixolab parameter using the Chopin+ and Wheatbug protocol.

			Chopin+ protocol[a]											Wheatbug protocol[b]
Crop	Damaged kernel replacement level	SSV (mL)	WA (%)	DDT (min)	Amplitude (Nm)	Stability (min)	C2 (Nm)	C3 (Nm)	C4 (Nm)	C5 (Nm)	α (Nm/min)	β (Nm/min)	γ (Nm/min)	CMC (%)
Common wheat	0	50 A	57.6 a	1.5 a	0.10 a	8.2 a	0.47 a	2.00 a	1.83 a	2.93 a	-0.08 a	0.55 a	-0.05 a	32 d
	1	47 B	57.9 a	1.7 a	0.09 a	8.0 a	0.48 a	1.99 a	1.79 ab	2.86 ab	-0.07 a	0.56 a	-0.04 a	34 d
	2.5	46 bc	57.6 a	1.5 a	0.10 a	6.6 b	0.45 ab	1.96 a	1.80 ab	2.87 ab	-0.08 a	0.57 a	-0.06 a	37 c
	5	44 c	57.3 a	1.4 a	0.09 a	5.5 c	0.42 b	1.89 b	1.72 bc	2.73 ab	-0.07 a	0.53 a	0.00 a	44 b
	10	41 d	57.6 a	1.9 a	0.08 a	4.1 d	0.37 c	1.82 c	1.71 bc	2.68 ab	-0.05 a	0.50 a	-0.01 a	49 ab
	20	32 e	57.8 a	1.9 a	0.08 a	2.5 e	0.28 d	1.71 d	1.64 d	2.54 b	-0.05 a	0.05 b	-0.01 a	54 a
	P (F)	**<0.001**	0.417	0.088	0.199	**<0.001**	**<0.001**	**<0.001**	**0.004**	**0.032**	0.120	**0.004**	0.063	**<0.001**
	sem[c]	1.3	0.44	0.31	0.02	0.22	0.02	0.03	0.05	0.16	0.02	0.14	0.03	3.0
Durum wheat	0	27 a	58.1 a	1.0 a	0.09 a	2.5 a	0.25 a	1.34 a	0.75 a	1.19 a	-0.05 a	0.46 a	-0.07 a	44 c
	1	24 b	58.1 a	1.0 a	0.08 a	2.3 a	0.24 ab	1.33 a	0.74 a	1.15 a	-0.05 a	0.40 a	-0.06 a	47 c
	2.5	20 c	57.9 a	1.0 a	0.09 a	2.1 ab	0.20 bc	1.31 ab	0.74 a	1.15 a	-0.05 ab	0.43 a	-0.08 a	52 b
	5	17 d	58.2 a	1.1 a	0.07 a	1.8 b	0.18 c	1.30 ab	0.77 a	1.17 a	-0.04 ab	0.43 a	-0.06 a	54 ab
	10	13 e	57.5 a	1.0 a	0.08 a	1.7 b	0.16 c	1.29 ab	0.77 a	1.21 a	-0.04 ab	0.43 a	-0.07 a	56 ab
	20	11 e	58.7 a	1.2 a	0.08 a	1.1 c	0.12 d	1.19 b	0.74 a	1.17 a	-0.03 b	0.43 a	-0.05 a	59 a
	P (F)	**<0.001**	0.057	0.608	0.316	**<0.001**	**<0.001**	**0.048**	0.602	0.739	**0.050**	0.927	0.098	**<0.001**
	sem[c]	1.0	0.63	0.27	0.02	0.29	0.03	0.07	0.07	0.09	0.01	0.11	0.02	2.5

Means followed by different letters are significantly different (the level of significance is shown in the table). Reported values are based on 2 replications. The experiment was carried out in the 2012-13 growing season.

[a] Mixolab parameters Chopin+ protocol: WA = water absorption, the amount of water required in dough development; DDT = Dough Development Time; stability = time of dough stability at constant temperature; amplitude = dough elasticity; C2 = protein strength; C3 = starch gelatinisation; C4 = hot gel stability; C5 = starch retrogradation in the cooling phase; α = protein network

weakening rate ; β = starch gelatinisation rate; γ = cooking stability rate. C2, C3, C4 and C5: end points of the corresponding mixing stages.

[b] Mixolab parameter “Wheatbug” protocol: CMC = Change in Mixolab Consistency

[c] sem = standard error of mean.

Table. 3

Correlation matrix between the sodium dodecyl sulphate sedimentation volume (SSV) and the Mixolab parameters[a], calculated separately for common and durum wheat.

Parameters	WA	DDT	Amplitude	Stability	C2	C3	C4	C5	α	β	γ	CMC	SSV
WA		0.056	0.198	-0.090	-0.082	-0.092	-0.116	-0.230	0.164	-0.026	0.088	0.186	-0.360
DDT	0.497		-0.852**	-0.295	-0.329	-0.333	-0.265	-0.261	0.457	-0.358	0.347	0.196	-0.183
Amplitude	0.447	0.478		0.573	0.547	0.575	0.536	0.499	-0.669	0.448	-0.588	-0.462	0.314
Stability	0.095	0.199	0.785*		0.985**	0.993**	0.981**	0.974**	-0.925**	0.854**	-0.549	-0.975**	0.910**
C2	-0.412	-0.089	0.442	0.851**		0.996**	0.970**	0.962**	-0.906**	0.929**	-0.497	-0.954**	0.935**
C3	-0.964**	-0.566	-0.369	0.089	0.583		0.983**	0.976**	-0.931**	0.899**	-0.552	-0.968**	0.929**
C4	-0.979**	-0.451	-0.544	-0.166	0.341	0.938**		0.993**	-0.936**	0.857**	-0.595	-0.984**	0.931**
C5	-0.971**	-0.307	-0.394	-0.060	0.432	0.921**	0.974**		-0.936**	0.842**	-0.605	-0.985**	0.956**
α	0.166	-0.272	-0.654	-0.930**	-0.910**	-0.297	-0.103	-0.237		-0.753*	0.606	0.938**	-0.830*
β	-0.874**	-0.632	-0.668	-0.403	0.111	0.836**	0.893**	0.828*	0.229		-0.345	-0.812*	0.892**
γ	0.545	0.527	-0.209	-0.422	-0.569	-0.602	-0.470	-0.452	0.428	-0.343		0.506	-0.471
CMC	0.672	0.130	-0.189	-0.615	-0.929**	-0.783**	-0.618	-0.713*	0.784*	-0.404	0.517		-0.928**
SSV	-0.241	0.157	0.594	0.909**	0.966**	0.402	0.175	0.301	-0.962**	-0.073	-0.422	-0.870**	

(*) = correlation significant at $P \le 0.05$; (**) correlation significant at $P \le 0.01$. Data reported in table are Pearson product-moment correlation coefficient.

The data with a gray background refer to durum wheat, while those with a white background refer to common wheat. The reported data for each crop refer to samples obtained from both the first and the second experiment in the 2012-13 growing season.

[a] Mixolab parameters Chopin+ protocol: WA = water absorption, the amount of water required in dough development; DDT = Dough Development Time; stability = time of dough stability at constant temperature; amplitude = dough elasticity; C2 = protein strenght; C3 = starch gelatinisation; C4 = hot gel stability; C5 = starch retrogradation in the cooling phase; α = protein network wakening rate ; β = starch gelatinisation rate; γ = cooking stability rate. C2, C3, C4 and C5: end points of the corresponding mixing stages. Mixolab parameter “Wheatbug” protocol: CMC = Change in Mixolab Consistency.

Figure

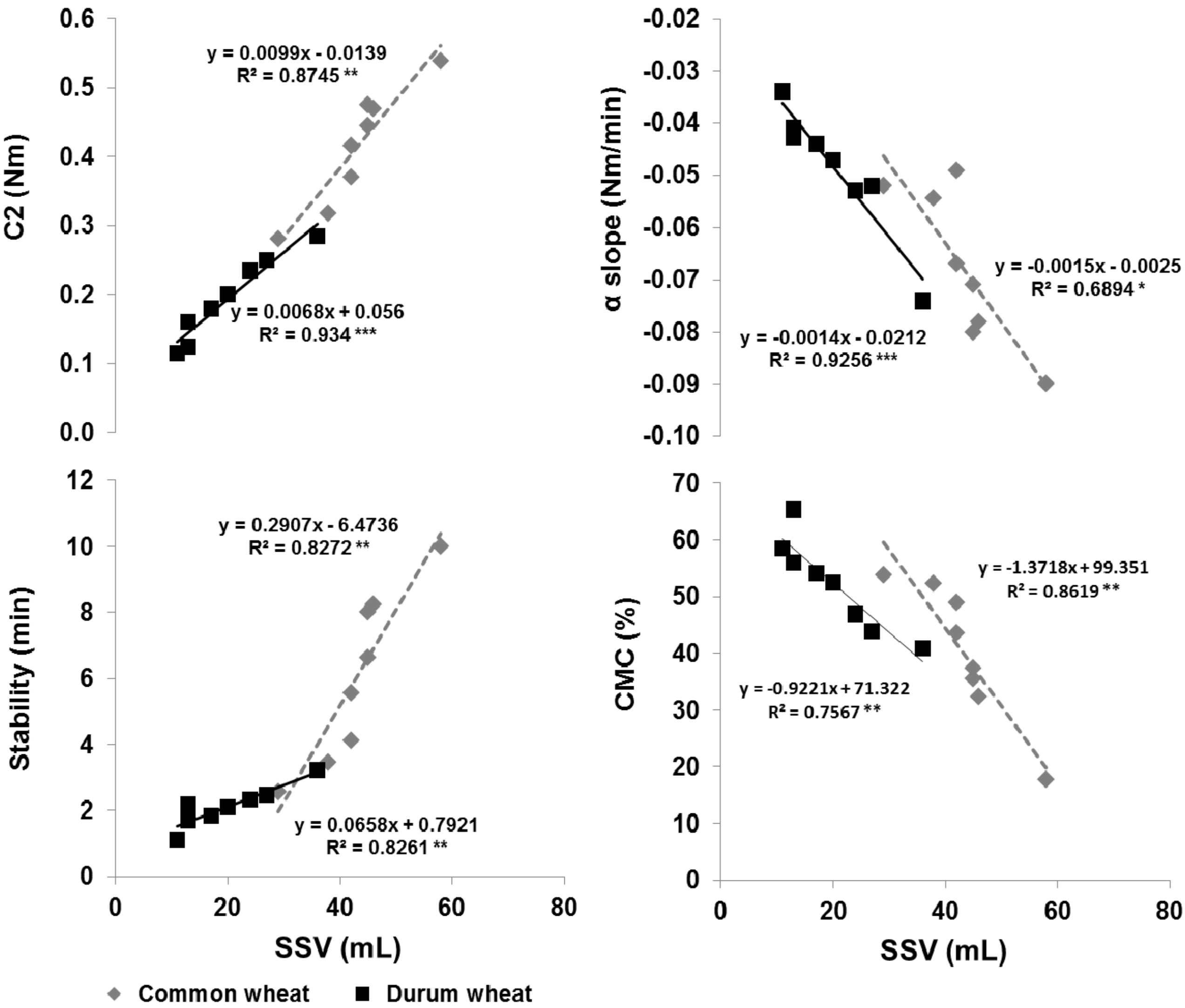

Figure. 1.

Relationship between sodium dodecyl sulphate sedimentation volume (SSV) and Mixolab stability, protein weakness (C2 point), protein network weakening rate (α slope) and Change in Mixolab Consistency (CMC) for common and durum wheat.

(*) = regression significant at P ≤ 0.05; (**) regression significant at P ≤ 0.01; (**) regression significant at P ≤ 0.001. R^2 = coefficient of determination of regression analysis. The reported data for each crop refer to samples obtained from both the first and the second experiment in the 2012-13 growing season.