

Combining intercropping with semiochemical releases: optimization of alternative control of *Sitobion avenae* in wheat crops in China

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Abstract

Overreliance on pesticides has large environmental and human health costs that compel researchers and farmers to seek alternative management tactics for crop pests. For insect pests, increasing crop species diversity via intercropping and using semiochemicals to alter local arthropod populations have separately proven effective at reducing pest densities. Here, we combine these two tactics in an effort to gain better control of *Sitobion avenae* (Fabricius) (Hemiptera: Aphididae), the English grain aphid, a major pest of cereal production worldwide. We conducted field experiments over 2 years testing the effectiveness of combining intercropping of wheat and oilseed rape with release of methyl salicylate (MeSA). We found that maximum and mean aphid densities were highest in wheat monocultures, significantly lower in intercropped plots and MeSA plots, and lowest when intercropping and MeSA release were combined by obtaining highest densities of predatory ladybeetles and parasitoids rates. Importantly, grain yield and quality showed a similar pattern: they were highest for combined intercropped/MeSA plots, intermediate in plots with intercropping or MeSA alone, and lowest in control monoculture plots. Our results suggest that combining these two tactics holds significant promise for improved management of aphid populations and emphasize the need to integrate alternative pest control approaches to optimize sustainable insect pest management.

Introduction

Efficiently controlling insect pest populations in agricultural crops is vital to optimizing yield and farm profitability. For cereal grains and many other crops produced in temperate climates, aphids are among the most challenging insect species to manage because their populations can increase quickly, their feeding can directly and indirectly damage the crop and influence yield, and they can vector yield-sapping pathogens (Van Emden & Harrington, 2007). Among aphid species, the English grain aphid [*Sitobion avenae* (Fabricius) (Hemiptera: Aphididae)] can be particularly problematic (Vickerman & Wratten, 1979; Hansen, 1995). This pest species attacks a range of small grains, feeding on phloem and spreading viruses (Van Em-

den & Harrington, 2007). In wheat [*Triticum aestivum* L. (Poaceae)] production, *S. avenae* can frequently cause economic damage, necessitating routine insecticide use. To reduce reliance on this pesticide use and associated economic, environmental, and health costs, researchers are exploring alternative, more sustainable strategies for managing pest populations.

A substantial body of literature has illustrated that insect pests are less problematic in areas with increased plant species diversity (e.g., Andow, 1991; Landis et al., 2000). However, increasing local plant species diversity may be difficult for many growers. A simple within-field solution to increasing local plant species diversity is intercropping, which can reduce insect pest populations compared with monocultures (Andow, 1991; Landis et al., 2000; Smith & McSorley, 2000). Oilseed rape, *Brassica napus* L. (Brassicaceae), is an economically important crop that has been widely used to examine the influence of crop diversification on abundance of arthropod pests and natural enemies

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(Hooks & Johnson, 2003). In China, oilseed rape intercropped with wheat has been demonstrated to significantly reduce the density of wheat aphids when compared to wheat monoculture (Wang et al., 2008, 2009).

In addition to intercropping, semiochemicals have also been shown to be useful for managing pest populations (James, 2003, 2005; James et al., 2004). Semiochemicals are natural chemical cues that mediate interactions between organisms such as plants and insects (Nordlund & Lewis, 1976). When attacked by herbivorous arthropods, many, if not most, plant species release volatile compounds that can act as repellents for herbivores or as attractants for natural enemies of herbivores, such as predators and parasitoids (Takabayashi & Dicke, 1996). Among semiochemicals, methyl salicylate (MeSA) is a volatile plant compound known to be very important for inducing resistance against pathogens and some herbivores (Shulaev et al., 1997). Methyl salicylate has also been demonstrated to be repellent to *Rhopalosiphum padi* (L.) and other cereal aphids (Pettersson et al., 1994; Glinwood & Pettersson, 2000; Ninkovic et al., 2003), whereas it has increased abundance in crops of predaceous beetles (e.g., *Coccinella septempunctata* L., *Stethorus punctum picipes* Casey), lacewings (e.g., *Chrysopa nigricornis* Burmeister, *Hemerobius* spec.), and bugs [e.g., *Deraeocoris brevis* (Uhler) and *Orius tristicolor* (White)] (James, 2003, 2005; James et al., 2004; Zhu & Papk, 2005).

Although both intercropping and semiochemicals can be effective in reducing aphid populations, to the best of our knowledge, thus far, these two tactics have been used independently. In this study, we combined the two tactics to determine whether the influences on aphid populations and crop yields of wheat intercropped with oilseed rape and MeSA release were additive or even synergistic.

Materials and methods

To test the combined influence of intercropping and semiochemical release on aphid populations, we conducted field experiments in two consecutive years at the experimental farm of Shandong Agricultural University, Shandong Province, China (36°09'N, 117°09'E). For these experiments, we used wheat variety 'Lumai 2' and oilseed rape variety 'Yuyou 5'. Both varieties are currently used commercially in China, for instance in provinces Shandong and Henan. For the semiochemical portion of the experiments, MeSA (≥99.7%) was obtained from the Chinese Academy of Military Medical Sciences.

Field experiments

The field experiments comprised four treatments: (1) wheat monocultures, (2) wheat intercropped with oilseed

rape, (3) MeSA release in wheat crop, and (4) wheat intercropped with oilseed rape and MeSA release. The four treatments were arranged in a completely randomized design with 10 × 10-m plots, and each treatment was replicated three times. Plots were bordered on all sides by 10-m-wide paths to decrease the possibility of natural enemies dispersing among treatments. Experimental fields were established in fall when wheat and oilseed rape were planted. Wheat was planted in 20-cm-apart rows at a rate of 120 kg ha⁻¹ on 11 and 15 October in 2007 and 2008, respectively. Oilseed rape was grown at the same time with wheat and kept in a greenhouse until seedlings were transplanted into the field plots on 10 November of each year. Seedlings had six true leaves at transplanting. Oilseed rape plants were spaced 40 cm apart within plots. Intercropped plots had eight rows of wheat, two rows of oilseed rape, and then the pattern repeated (Wang et al., 2009). All treatments were fertilized with 150:50:25 (NPK) kg ha⁻¹, and no insecticides or herbicides were used in the whole experimental area. Plots were irrigated twice in each year, once during seedling establishment and once during seed fill.

To release MeSA, we used a slow-release apparatus based on a small cylindrical plastic box (inner diameter 6.5 cm, 4 cm tall) containing a sponge. The chemical was injected into the sponge inside the box, which had four 2-cm holes drilled through the top. The plastic boxes were attached to crabsticks and spaced 1 m above the ground, set at the center of each plot, one box for each plot. Boxes emitted doses of 120 mg MeSA m⁻² per week, which was based on a previous study (Pettersson et al., 1994). The first application of MeSA was made at the jointing stage on 16 and 17 April, in 2008 and 2009, respectively, and subsequently applied every 7 days, for four times in 2008 and five times in 2009.

Sampling of wheat aphids and natural enemies

To evaluate the influence of the four treatments on insect populations, we sampled plots for *S. avenae* and its predators and parasitoids. To sample *S. avenae*, we used a 'Z-shaped' sampling pattern in which 10 sampling sites were selected within each plot. At each sampling site, we randomly selected 10 wheat tillers and counted the *S. avenae* on all the tillers (10 sites, 10 tillers per site: 100 tillers per plot). For predatory lady beetles (i.e., *C. septempunctata*, *Harmonia axyridis* Pallas, and *Propylaea japonica* Thunberg), we counted all stages of beetles found on wheat plants within five quadrates randomly positioned within each plot, each quadrate was 0.2 m². For aphid parasitoids (i.e., *Aphidius avenae* Haliday and *Aphidius gifuensis* Ashmead), we counted the aphid mummies on the same 100 wheat tillers mentioned above for *S. avenae*. Parasitism

rates (derived from mummy counts) were calculated at the end of each sampling period. Insect species were sampled at 3-day intervals from 16 April to 22 May in 2008 and from 17 April to 23 May in 2009 (from wheat jointing stage to mature stage).

Statistical analysis

Population densities of insect species were compared among treatments using analysis of variance (ANOVA), followed by comparison of means using Duncan's multiple range test. In both years, yield (tonnes ha⁻¹) and thousand grain weight (TGW; g) were calculated for each treatment. These data were analyzed by one-way ANOVA followed by Duncan's multiple range test. Effects of years and treatments and the possible interaction between wheat-oilseed rape intercropping and MeSA release were analyzed using general linear model (GLM) procedure. Where necessary, the data used in ANOVA and GLM were transformed using \sqrt{x} to meet assumptions of normality (SPSS for Windows, version 16.0).

Results

Maximum *Sitobion avenae* densities

In both 2008 and 2009, significant differences were detected in maximum *S. avenae* densities per 100 tillers between the control and the three other treatments (2008: $F_{3,8} = 107.64$, $P < 0.01$; 2009: $F_{3,8} = 44.08$, $P < 0.01$; Table 1). In both years, aphid maximum densities were lowest in the combined intercropping-MeSA plots compared to the other three treatments. No significant difference was detected between intercropping-alone and MeSA-alone plots.

Aphid densities, predatory lady beetles, and parasitoids of *Sitobion avenae*

In both 2008 and 2009, significant differences were detected in the mean numbers of aphids per 100 tillers

Table 1 Effects of treatments on mean (\pm SE) maximum *Sitobion avenae* densities during 2008 and 2009

Treatment	Year	
	2008	2009
Control	2 483 \pm 77a	2 218 \pm 76a
Wheat-oilseed rape intercropping	1 120 \pm 77b	1 145 \pm 83b
MeSA release in wheat crop	1 320 \pm 71b	1 361 \pm 67b
Intercropping with MeSA release	761 \pm 60c	935 \pm 106c

MeSA, methyl salicylate.

Means in the same column followed by different letters are significantly different (Duncan's multiple range test: $P < 0.05$).

between the control and the other three treatments (2008: $F_{3,8} = 97.58$, $P < 0.01$; 2009: $F_{3,8} = 90.11$, $P < 0.01$; Figure 1A). The average densities of wheat aphids were highest in control plots and lowest in combined intercropping-MeSA plots. No significant difference in aphid densities was detected between MeSA and intercropped plots.

Consistent with our results for aphid densities, predatory lady beetles were most abundant in combined intercrop-MeSA plots, and these populations were significantly more abundant than intercropped-alone or MeSA-alone plots, which contained in turn significantly more predatory lady beetles than monoculture controls (2008: $F_{3,8} = 15.43$, $P < 0.01$; 2009: $F_{3,8} = 23.59$, $P < 0.01$; Figure 1B). Mean parasitism rates of *S. avenae* showed a pattern similar to that of lady beetles (2008: $F_{3,8} = 11.22$, $P < 0.01$; 2009: $F_{3,8} = 18.32$, $P < 0.01$; Figure 1C).

Yield and quality

In both years, yield and TGW differed significantly between treatments (yield, 2008: $F_{3,8} = 15.32$, $P < 0.01$; 2009: $F_{3,8} = 11.39$, $P < 0.01$; TGW, 2008: $F_{3,8} = 15.94$, $P < 0.01$; 2009: $F_{3,8} = 9.51$, $P < 0.01$; Table 2). Yield and TGW in combined intercrop-MeSA plots were significantly higher than in the other three plots. However, in 2008, no significant difference was detected among the other three treatments in yield, whereas in 2009, no significant difference in TGW was detected between intercropped and monoculture plots.

Two-factor effects

In addition to within-year analyses, we also compared results across years with two-factor ANOVA (Table 3). Between the 2 years, yield and aphid density were not significantly different, but we did detect significant differences in the number of lady beetles, parasitism rate, and TGW. Other than for parasitism rates, no significant difference was detected in interactions between year and treatments (Table 3). Between the 2 years, we also detected significant interactions between intercropping and MeSA release treatment for aphids, but no significant interaction was detected for lady beetles, parasitism rates, yields, and TGWs (Table 4).

Discussion

Our results support the combined power of intercropping and semiochemical release. Control of aphids attained when the two approaches were combined was significantly improved compared to intercropping and MeSA release individually. In fact, the effect on aphid control was so strong that the two tactics seemed to act in synergy. The mechanism of this improved control might have been due

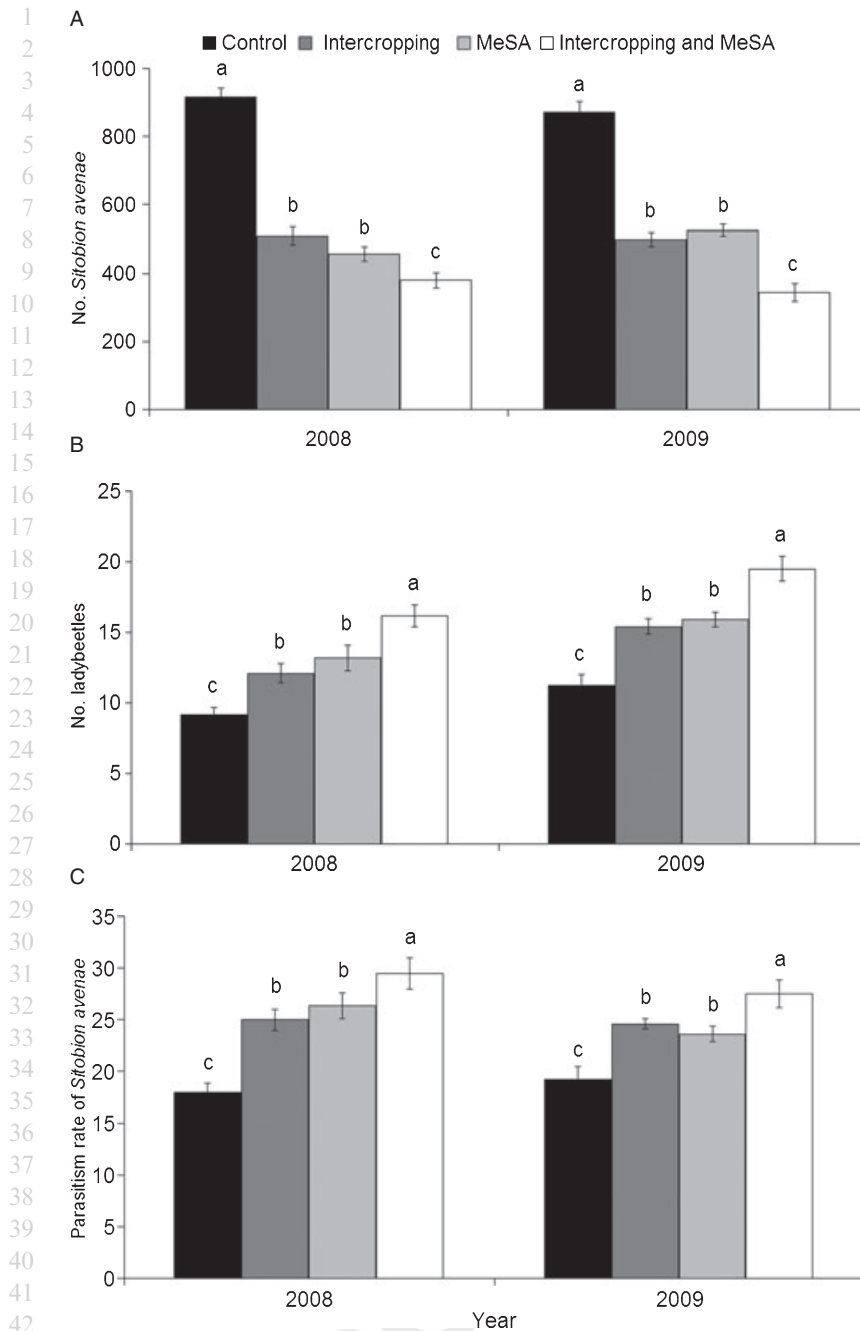


Figure 1 Field experiment testing the influence of four treatments (wheat monoculture, wheat intercropped with oilseed rape, methyl salicylate (MeSA) release in wheat crop, and the combination of MeSA and wheat–oilseed intercrop) on insect populations (mean number per 100 tillers \pm SE). (A) *Sitobion avenae*, (B) lady beetles, and (C) *S. avenae* parasitized. Bars capped with different letters within a year are significantly different (Duncan's multiple range test: $P < 0.05$).

to two factors. First, MeSA may have been directly repellent to *S. avenae*, reducing initial aphid colonization early in the season (Pettersson et al., 1994). Alternatively, MeSA may have increased the mobility of aphids, enhancing their exposure to predators (Griffiths et al., 1985; Sunderland et al., 1986). Increased mobility may also have reduced time spent in optimal feeding sites, preventing populations from growing as quickly as they could (Wiktelius, 1989). Second, combined MeSA and intercropping

plots may have developed larger populations of natural enemies that killed more aphids. This notion was supported by the greater numbers of lady beetles and parasitized aphids in combined plots (Figure 1B and C). These natural enemies could have been attracted to plots by MeSA (Zhu et al., 1999; Kean et al., 2003; James & Price, 2004; Prinsloo et al., 2007) and then maintained within the combined plots by alternative prey provided by intercropped plants. As for some other natural enemy species

Table 2 Mean (\pm SE) yield and thousand grain weight (TGW) of wheat in treatment plots

Treatment	Yield (tonnes ha ⁻¹)		TGW (g)	
	2008	2009	2008	2009
Control	5.30 \pm 0.11c	5.39 \pm 0.09b	31.52 \pm 1.10c	30.09 \pm 1.55c
Wheat–oilseed rape intercropping	5.88 \pm 0.19b	5.63 \pm 0.10b	36.02 \pm 1.19b	33.78 \pm 0.83bc
MeSA releases in wheat crop	6.06 \pm 0.12b	5.72 \pm 0.18b	37.35 \pm 0.38b	34.05 \pm 0.38b
Intercropping with MeSA releases	6.65 \pm 0.14a	6.42 \pm 0.13a	41.38 \pm 1.18a	38.7 \pm 1.41a

MeSA, methyl salicylate.

Means in the same column followed by different letters are significantly different (Duncan's multiple range test; $P < 0.05$).

Table 3 F-statistics for effect of year and treatment on abundance of *Sitobion avenae*, lady beetles, parasitism rate, yield, and thousand grain weight (TGW)

Source of variation	d.f.	F-value				
		Aphids	Lady beetles	Parasitism rate	Yield	TGW
Treatment	3	190.84**	38.19**	71.04**	26.04**	24.44**
Year	1	0.44ns	31.93**	196.32**	3.42ns	9.90**
Treatment*year	3	2.82ns	0.33ns	6.62**	0.97ns	0.27ns

** $P < 0.01$, ns: $P > 0.05$.

Table 4 F-statistics for effect of wheat–oilseed rape intercropping and methyl salicylate (MeSA) releases on abundance of *Sitobion avenae*, lady beetles, parasitism rate, yield, and thousand grain weight (TGW)

Year	Source of variation	F-values				
		Aphids	Lady beetles	Parasitism rate	Yield	TGW
2008	Intercropping	99.68*	16.03*	19.48*	16.99*	13.31*
	MeSA	147.01*	30.25*	12.85*	29.68*	15.04*
	Intercropping*MeSA	46.06*	1.29ns	1.33ns	0.06ns	0.18ns
2009	Intercropping	139.81*	31.14*	19.88*	12.55*	17.56*
	MeSA	113.66*	39.47*	33.47*	18.01*	30.21*
	Intercropping*MeSA	16.87*	1.65ns	1.62ns	2.69ns	0.05ns

* $P < 0.05$, ns: $P > 0.05$.

(e.g., spiders, lacewings, and syrphids), their numbers were too small to have any significant effect on the aphids. On oilseed rape plants, we found at least two other aphid species [*Myzus persicae* Sulzer and *Lipaphis erysimi* (Kaltenbach)], which could have served as alternative hosts for a parasitoid (i.e., *A. gifuensis*) and predators (i.e., *P. japonica*, *H. axyridis*, and *C. septempunctata*) during the early stage of wheat growth, because these two aphid species reach their peaking period about 10 days earlier than that of *S. avenae* in intercropping plots.

6 Yield and TGW were significantly higher in combined intercropped and MeSA plots than in plots of the other three treatments in 2008 and 2009. In 2009, we found no

significant difference in yields among the other three treatments and no difference in TGW between the control and intercropping plot. Differences in yield and TGW may have been due to various degrees of aphid exposure that is caused by differential populations of natural enemies in different treatment plots.

Increased plant species diversity in agroecosystems can improve abundance of natural enemies by providing them nectar, pollen, or alternative prey, which can prolong their lives and increase the number of herbivores they can kill in a lifetime (Pemberton & Lee, 1996; Ruhren & Handel, 1999; Cuautle & Rico-Gray, 2003). The flowering period of oilseed rape in Shandong province is from 15 to 30

1 April, more than 8 days before the *S. avenae* population
 2 peak. Therefore, oilseed rape likely provided alternative
 3 resources, such as floral nectar and/or pollen, that bene-
 4 fited natural enemies and facilitated improved pest con-
 5 trol. As mentioned earlier, we found other aphid species
 6 that could have helped support natural enemies in inter-
 7 crop–MeSA plots.

8 Although wheat–oilseed rape intercropping enhanced
 9 the abundance of predators and parasitoids, they may have
 10 failed to suppress the initial aphid colonization of wheat,
 11 because at the time of transplant of the young small oilseed
 12 rape plants and the wheat seedlings, there was abundant
 13 bare soil in those plots. Aphids locate potential host plants
 14 by contrasting the plant with the soil background (Ken-
 15 nedy et al., 1959, 1961), so the appearance of bare soil may
 16 have encouraged aphids to preferentially colonize wheat in
 17 wheat–oilseed rape intercropping fields. Alternatively, as a
 18 repellent to *S. avenae* and an attractant to its natural ene-
 19 mies, MeSA release in intercropped plots may have offset
 20 initial aphid colonization, enhancing the killing action of
 21 predators and parasitoids.

22 Our results strongly support the efficacy of combining
 23 intercropping and semiochemical release to attain
 24 improved pest control in small grain production. Whereas
 25 these two tactics have been shown to improve pest control
 26 independently, their combined power is even greater.
 27 Based on the simplicity of each tactic, there would appear
 28 to be only a few minor barriers to adoption by growers.
 29 Given widespread adoption, integrating these two tactics
 30 would appear to hold great promise for improving sustain-
 31 able pest management while reducing reliance on insecti-
 32 cides.

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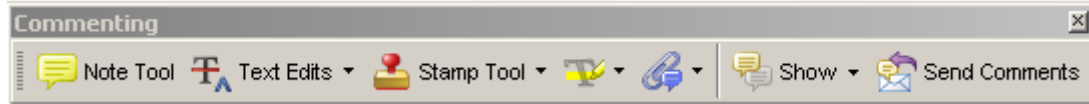
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Adobe Acrobat Professional or Acrobat Reader (version 7.0 or above) is required to e-annotate PDFs. Acrobat 8 Reader is a free download: <http://www.adobe.com/products/acrobat/readstep2.html>

Once you have Acrobat Reader 8 on your PC and open the proof, you will see the Commenting Toolbar (if it does not appear automatically go to Tools>Commenting>Commenting Toolbar). The Commenting Toolbar looks like this:



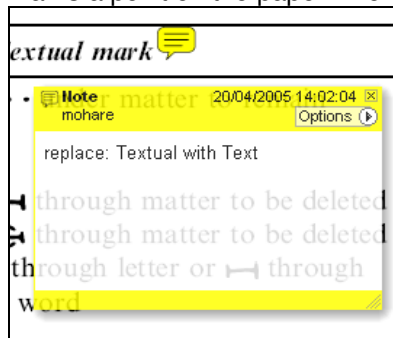
If you experience problems annotating files in Adobe Acrobat Reader 9 then you may need to change a preference setting in order to edit.

In the “Documents” category under “Edit – Preferences”, please select the category ‘Documents’ and change the setting “PDF/A mode:” to “Never”.



Note Tool — For making notes at specific points in the text

Marks a point on the paper where a note or question needs to be addressed.

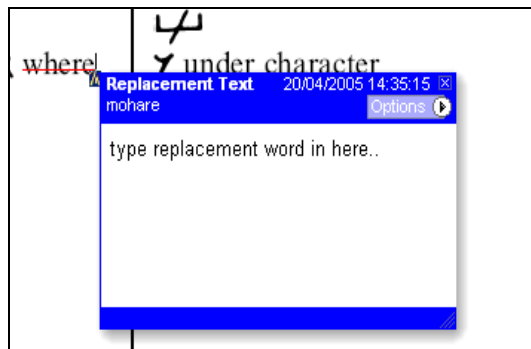


How to use it:

1. Right click into area of either inserted text or relevance to note
2. Select Add Note and a yellow speech bubble symbol and text box will appear
3. Type comment into the text box
4. Click the X in the top right hand corner of the note box to close.

Replacement text tool — For deleting one word/section of text and replacing it

Strikes red line through text and opens up a replacement text box.

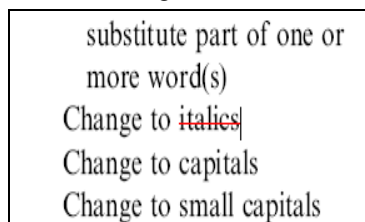


How to use it:

1. Select cursor from toolbar
2. Highlight word or sentence
3. Right click
4. Select Replace Text (Comment) option
5. Type replacement text in blue box
6. Click outside of the blue box to close

Cross out text tool — For deleting text when there is nothing to replace selection

Strikes through text in a red line.



How to use it:

1. Select cursor from toolbar
2. Highlight word or sentence
3. Right click
4. Select Cross Out Text

Approved tool — For approving a proof and that no corrections at all are required.

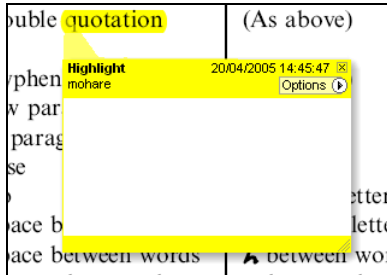


How to use it:

1. Click on the Stamp Tool in the toolbar
2. Select the Approved rubber stamp from the 'standard business' selection
3. Click on the text where you want to rubber stamp to appear (usually first page)

Highlight tool — For highlighting selection that should be changed to bold or italic.

Highlights text in yellow and opens up a text box.

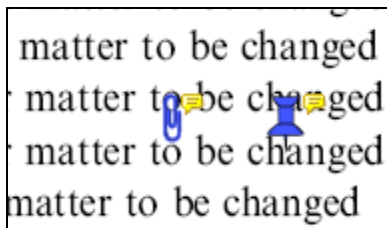


How to use it:

1. Select Highlighter Tool from the commenting toolbar
2. Highlight the desired text
3. Add a note detailing the required change

Attach File Tool — For inserting large amounts of text or replacement figures as a files.

Inserts symbol and speech bubble where a file has been inserted.

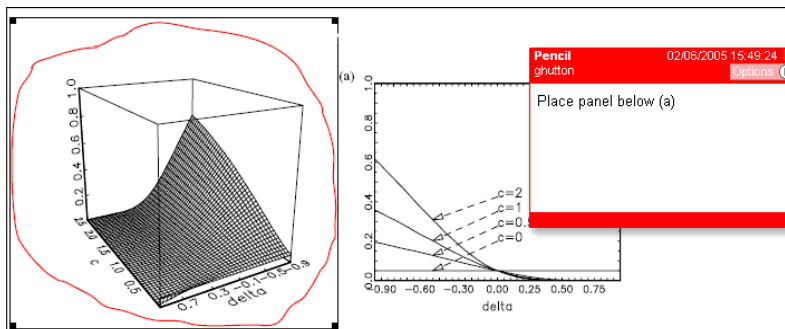


How to use it:

1. Click on paperclip icon in the commenting toolbar
2. Click where you want to insert the attachment
3. Select the saved file from your PC/network
4. Select appearance of icon (paperclip, graph, attachment or tag) and close

Pencil tool — For circling parts of figures or making freeform marks

Creates freeform shapes with a pencil tool. Particularly with graphics within the proof it may be useful to use the Drawing Markups toolbar. These tools allow you to draw circles, lines and comment on these marks.



How to use it:

1. Select Tools > Drawing Markups > Pencil Tool
2. Draw with the cursor
3. Multiple pieces of pencil annotation can be grouped together
4. Once finished, move the cursor over the shape until an arrowhead appears and right click
5. Select Open Pop-Up Note and type in a details of required change
6. Click the X in the top right hand corner of the note box to close.

Help

For further information on how to annotate proofs click on the Help button to activate a list of instructions:

